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Molluscan Research

Australasian Abalone

Edited by S.A. Shepherd, P. E. McShane and F.E. Wells

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THE AUSTRALASIAN SYMPOSIUM ON ABALONE FISHERIES, CULTURE AND BIOLOGY
WELLINGTON, NEW ZEALAND, October 1996.

INTRODUCTION

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This special issue of Molluscan Research contains 19 papers given at the first regional abalone symposium in Wellington, New Zealand in October 1996. In the Introduction to 'Progress in Abalone Research' containing the papers from the 1994 2nd International Abalone Symposium in Hobart, Day and Shepherd (1995) suggested that the most fertile topics for future research in wild fisheries for abalone were likely to be:

- the use of cultured larvae to study the early life history
- larval dispersal and larval availability to reefs
- the relation between egg production and later recruitment
- the aging of abalone shells, and
- the effect of fishing on abalone aggregations.

It is pleasing to see that many of these topics have been vigorously pursued in the short time that has elapsed since the Hobart meeting. On the first topic we have seven papers on the ecology of larvae on settlement or of post-larvae after settlement. **Roberts and Nicholson** show that larval settlement is not a single process, but has two distinct elements – attachment and metamorphosis – each of which may have its own suite of biochemical cues. **Daume *et al.*** demonstrate the dependence of post-larvae on crustose coralline algae (CCA) for food in the first week or two before they begin feeding on diatoms. **Kitting and Morse** further explore the relation between post-larvae and CCA from the point of view of the algal host (a decrease in epiphytes and a stimulus for growth) and speculate on the evolution of this extraordinary symbiotic mutualism. In some elegant experiments **Takami *et al.*** show that CCA rapidly become inadequate for a growing post-larva, and must be supplemented by diatoms to promote rapid growth. **Moss** pursues the theme from a maricultural point of view and determines the optimal size at which the diet of a tiny abalone should switch from diatoms to macro-algae or artificial food. The last two of this septet of papers used cultured larvae to study the survival of settlers in the wild: **Preece *et al.***

in the context of stock enhancement (a subject of increasing interest wherever stocks are declining) and **Naylor and McShane** in an assessment of sources of variation in post-settlement survival. The latter paper described a simple device for simulating the water flows encountered in subtidal habitats.

On the second topic **Rodda *et al.*** deployed larval collectors for 3 years to measure the recruitment of larvae above natural reefs. This study has provided insight into larval dispersal and the timing of episodic settlement on reefs. Progress will be even more when the post-larvae of the five sympatric species of abalone can be identified to species, and the relation between larval availability and subsequent recruitment to reefs is clarified.

The third topic has been addressed obliquely in two studies that examined patterns of recruitment in two species in superficially similar habitats – studies which incidentally raise intriguing questions about the different life history strategies that have evolved in different conditions. **Wells and Keesing** found that weak recruitment can be offset by a very fast juvenile growth which reduces the window

of vulnerability to predators. In contrast, **Andrew *et al.*** found apparently much stronger recruitment, which maintained a stable fishery despite a high exploitation rate. Both studies are reminders of the perils of extrapolating conclusions from one abalone fishery to another.

The fourth topic on shell-aging has been advanced, in terms of methodology and practice, in five papers. **Hawkes *et al.*** establish the use of manganese as a time-stamp for marking shells for validation of shell-aging, and **Shepherd *et al.*** in a quartet of papers describe the rate of ring deposition in two Mexican species and two Australian ones. Each species described has a different pattern of ring deposition, and sometimes there is variation within a species, and sometimes variation due to confounding factors such as endobionts and shell abrasion. This topic will certainly attract more attention as biologists seek to apply age-structured models to abalone fisheries.

The importance of the fifth topic on effects of fishing is now increasingly recognised in the light of recent work on the fertilisation success in free-spawning invertebrates. In an unusual approach **McShane** shows how the perceptions of divers regarding aggregation sizes and recruitment shape their selective fishing behaviour – which may ultimately have consequences for recruitment to the fishery. Clearly we need a better understanding of the interaction between diver behaviour (the predator) and the behavioural biology of the prey abalone.

Of course some papers will always fall outside the best-laid prescriptions of mice and men but are still of great interest. **Hooker *et al.*** consider the growth of *Haliotis iris* near its northern limit, and **Friedman *et al.*** describe a fungal disease which has long been known to infest New Zealand abalone.

Finally, and fittingly, **Schiel** has given a useful review of research on New Zealand abalone with a critique, aptly acerbic, of research in the fledgling abalone mariculture industry.

The topical framework employed in this introduction, while not intended to be prescriptive, will nevertheless, we believe, be valid for research in wild fisheries for some years to come. Yet if we were to venture to predict a topic that will soon become significant it would be this. Given the increasing emphasis on ecosystem, as opposed to single species, management of coastal waters (Larkin 1996) where exploitation of species at different levels of the food web, from algae to primary, to secondary consumers, is ever increasing, attention must be given to the relations between abalone and all other species which share its niche. This is an ambitious order because the niche of an abalone changes in scale from the tiny (the CCA habitat) to the reef at a scale of metres, to clusters of reefs (the metapopulation dimension) at a scale of kilometres, and embraces macro-algae in their structural role as well as for food, and predators, competitors and parasites. We look forward to further Australasian studies which emphasize the ecological interactions between abalone and other reef-dwelling organisms.

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Acknowledgements

All papers were reviewed in accordance with the policy of Molluscan research and we thank the 30 or so referees for their contributions to the work. Our task would have been almost impossible and this volume impoverished without their advice.

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Effects of post-larval abalone (*Haliotis rubra*) grazing on the epiphytic diatom assemblage of coralline red algae

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Abstract

Larvae of the abalone *Haliotis rubra* were settled on pieces of the non-geniculate coralline red alga *Phymatolithon repandum*. We measured a mean growth-rate of ca. 30 µm/day, 11 days after larval settlement which stayed constant throughout the experiment. The diatom assemblage on the surface of the alga was studied for 53 days after larval settlement using scanning electron microscopy. Two species of the genus *Cocconeis* were found to dominate the diatom assemblage on the surface of *P. repandum*. Up to 18 days after larval settlement, the diatom population increased exponentially. Eighteen days after settlement, post-larval abalone started to graze on the diatom *Cocconeis scutellum* leaving the bottom valves of the diatoms on the surface of *P. repandum*. As a result, the diatom population decreased markedly. The outermost cells and polysaccharide layer of *P. repandum* were often missing but we did not find any grazing marks on the thallus surface up to 53 days post-settlement. We discuss the hypothesis that epithallial cell sloughing of non-geniculate coralline red algae is an intrinsic mechanism to reduce microscopic epiphytes and may interact closely with grazing by post-larval abalone. We concluded that the post-larvae must find an additional food source derived from the NCA before grazing on the diatoms, because of the high growth-rate during the first and second week of rearing and the constancy throughout the experiment.

Introduction

The relationship between abalone and non-geniculate coralline red algae (NCA) (Corallinales, Rhodophyta) is well known. Abalone larvae settle on NCA (Morse & Morse 1984; 1988; Morse 1990; 1991) and remain in the coralline habitat until they are about 6 mm in shell length (Shepherd & Daume 1996). Post-larval abalone are known to feed on benthic diatoms (Kawamura *et al.* 1995). Diatoms occurring on artificial plates have also been used as the initial food source in commercial abalone hatcheries. Recent interest has focused on settlement and feeding experiments of post-larval abalone on species of benthic diatoms (Kawamura & Kikuchi 1992; Kawamura & Takami 1995; Kawamura *et al.* 1995; Matthews & Cook 1995; Kawamura 1996). Despite the importance of diatoms in abalone feeding ecology, the naturally occurring diatom assemblage on the NCA surface has never been studied in detail.

Molluscan herbivores have a major effect on the algal community by feeding on the sporelings of macroalgae (Dayton 1975; Menge 1976; Underwood & Jernakoff 1981). If herbivores are removed, the macroflora increases. This effect has been well documented in the past (Southward 1964; Dayton 1971) and later tested at the microflora level (Nicotri 1977; Underwood 1984) however, the influence of post-larval abalone grazing on the diatom assemblage of NCA surfaces has not been investigated.

Phymatolithon repandum (Foslie) Wilks and Woelkerling 1994 and other species of NCA frequently slough epithallial cells, and this has been interpreted as an antifouling mechanism which

inhibits algal spore settlement (Masaki *et al.* 1984; Johnson & Mann 1986; Keats *et al.* 1993; 1997). The effects of epithallial cell sloughing on the diatom assemblage of NCA surfaces is described in this study.

Herbivores like *Cellana* spp., *Acmaea* spp., *Notoacmaea* spp., *Patella* spp. as well as *Haliotis* spp. are known to leave grazing marks produced by the specialized teeth of their radula while feeding on NCA (Clarkson & Shepherd 1985; Padilla 1985; Garland *et al.* 1985). Garland *et al.* (1985) described grazing marks of 6 week old post-larvae which were 1–2 cells deep. It is unknown however, if post-larval abalone younger than 6 weeks leave grazing marks on the thallus surface.

In this paper we describe:

(1) the distribution of the dominant diatom species on the surface of the NCA *Phymatolithon repandum*;
(2) the effect of post-larval abalone grazing on the dominant diatom species from day of larval settlement to 53 days after settlement;
(3) cell sloughing on different growth-forms of *P. repandum* and the effect it has on the diatom assemblage; and
(4) the radula structure and teeth arrangement of post-larval abalone.

Materials and Methods

Sampling

Small rocks encrusted with NCA were collected from a boulder habitat in front of the Gloucester Reserve, Williamstown, Victoria, Australia (37° 53'S, 144° 55'E) at 3– 4 m depth. Williamstown is located at the north end of Port Phillip Bay and is close to Port Melbourne and the mouth of the Yarra River. Only the blacklip abalone, *Haliotis rubra* Leach 1814 is present here. *Phymatolithon repandum* is the dominant NCA species at this locality (unpublished data) and occurs with two different growth-forms (encrusting and warty, see Woelkerling *et al.* 1993). Pieces (ca. 1 cm^2) of each growth-form were removed from the rocks with a razor blade or chisel. Each replicate for the diatom study and the experiment was obtained from a different plant to ensure independence.

Distribution of the dominant diatoms on *P. repandum*

Pieces of the encrusting and warty growth forms were freshly collected and air dried after sampling to study the composition and distribution of diatoms on the thallus surface. The samples were mounted on aluminium stubs with 'Fotobond' acrylic adhesive (Agfa-Gevaert Ltd). The stubs were sputter-coated with gold before viewing in a Siemens ETEC Autoscan microscope at 20 kV to determine the frequency of diatom occurrence on the two growth-forms of *P. repandum*. For each sample, the presence or absence of diatom species was recorded at a magnification of 1200x in 20 fields of view.

Effect of post-larval grazing

Larvae of the abalone *Haliotis rubra* obtained from a hatchery (Cheetham Salt, Lara, Victoria, Australia) were allowed to settle onto surfaces of *Phymatolithon repandum*. Two pieces of *P. repandum* (ca. 1 cm^2) representing the encrusting and warty growth forms were placed in each of 24 sterile glass jars with 300 ml UV sterilised sea water. Jars were kept at 15° C, 3000 lux with a 12 h L:D photo cycle. One hundred larvae competent to settle were added to each of 18 jars. Six jars, without larvae and only the warty growth-form, were used as a control. The shell length of four randomly chosen larvae was measured before being added to the jars. Three to four larvae were measured 11, 18 and 53 days after settlement to calculate their growth-rate.

Less than 20 larvae/cm^2 were maintained on each piece of NCA. Abalone were observed moving over the NCA surface through a dissecting microscope, showing side to side scraping by the radula and judged to be actively feeding.

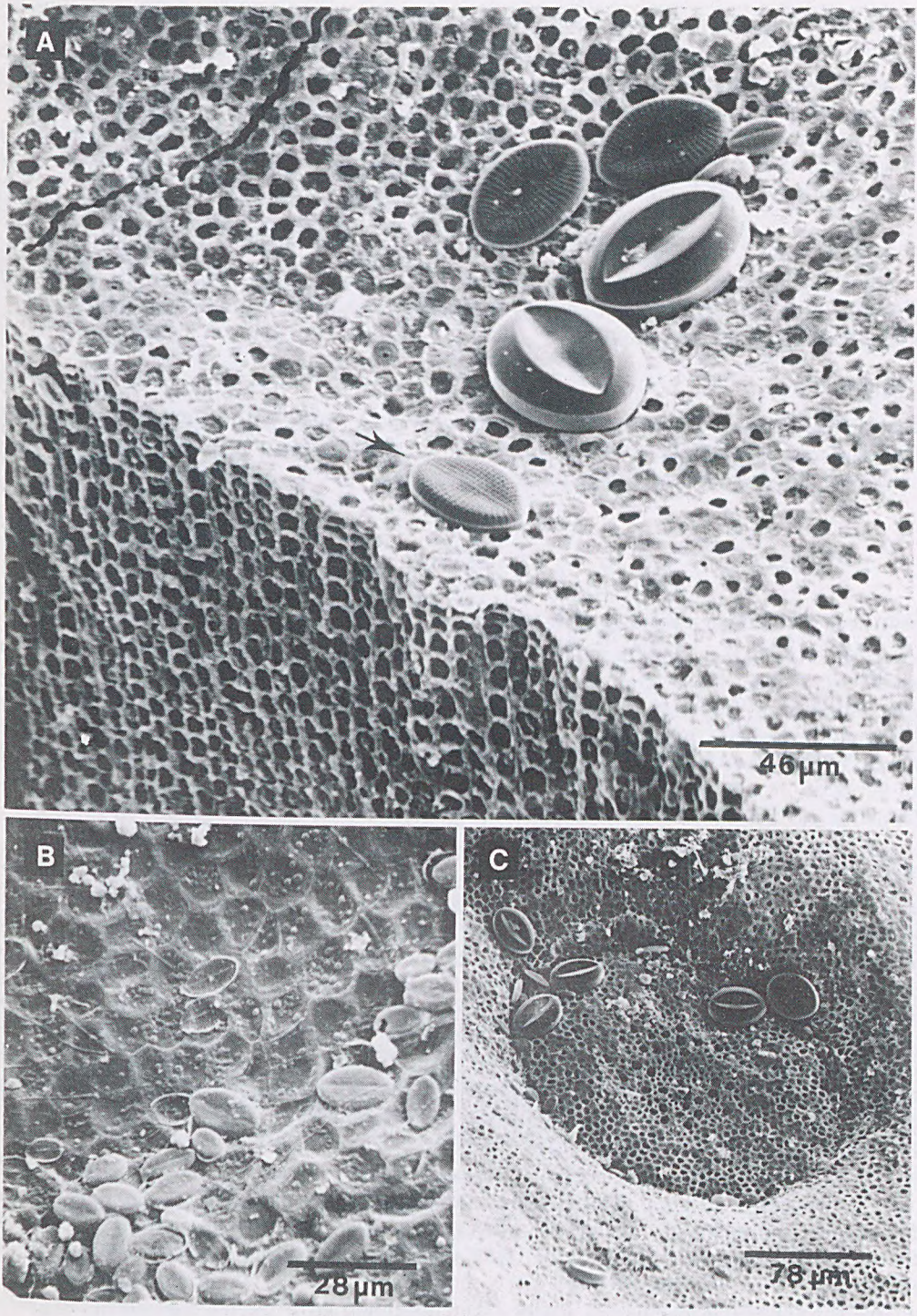

Figure 1. Diatoms (*Cocconeis* spp.) on the surface of *Phymatolithon repandum*. A: Two species of *Cocconeis*: *Cocconeis scutellum* (arrow) and *Cocconeis sp.* B: Diatoms occur in a patchy distribution. C: Diatoms proliferate around conceptacles.

Diatom distribution under grazing pressure and cell sloughing of NCA

Pieces of both NCA growth-forms with post-larvae and pieces from the control jars (without post-larvae) were subsampled randomly 4, 11, 18, 30 and 53 days after larval settlement. The subsamples were air dried and treated for the scanning electron microscopy (SEM) study as described above. For each sample, the presence or absence of diatom species and cell sloughing was recorded at a magnification of 1200x and 150x respectively in 20 fields of view. The mean frequency (± standard error) of diatom species and cell sloughing was calculated.

Abalone radula

To study the post-larval abalone radula, the shell was removed, the tissue of the whole animal dissolved in 6% sodium hypochlorite overnight, washed in distilled water, allowed to dry and then prepared for SEM as described above.

Data analysis

Statistical analyses were carried out using the STATISTICA (Statsoft Inc. 1995) computer package. One-way and two-way ANOVAs were carried out on untransformed data (number of fields with *Cocconeis* spp.) followed by Tukey's multiple comparison test.

Results

Distribution of the dominant diatoms on *P. repandum*

During the period of the study (October to December 1995) only pennate diatoms were observed on the surface of *P. repandum*. The genus *Cocconeis* was represented by two species (*Cocconeis scutellum* (Ehrenberg) Boyer 1927; *Cocconeis sp.*) (Fig. 1A) that dominated the diatom assemblage. On all samples examined, they formed 90–100% of the diatom population with a size range from 10 to 40 µm. We observed a low frequency (5–10%) of diatoms on freshly sampled pieces of both growth-forms of *P. repandum*. All diatoms were found in a patchy distribution (Fig. 1B) and proliferated in cavities and around conceptacles (Fig. 1C).

Effect of post-larval grazing

The frequency of *Cocconeis* spp. on the surface of *P. repandum* grazed by post-larval abalone changed during the period of study (Fig. 2). Between four and 11 days after abalone larvae settled on the NCA surface, the frequency of *Cocconeis* spp. increased exponentially on both growth-forms of NCA but not on the controls where larvae were absent. *Cocconeis* spp. reached a maximum frequency of 80% on the encrusting growth-form 11 days after larval settlement (Fig. 3A). There was a highly significant difference in frequency of *Cocconeis* spp. between the control and the 2 treatments with post-larval abalone 11 and 18 days after settlement (2-way ANOVA, Treatment x Age Interaction, $p<0.001$). However, there was no significant difference in diatom frequency between the warty and the encrusting growth-form throughout the experiment (2-way ANOVA, $p=0.24$).

After 18 days, the *Cocconeis* population decreased on both treatments with post-larval abalone (Fig. 2). On the grazed surfaces, raphe-bearing valves (bottom valves) of *C. scutellum*, ca. 20 µm in length, appeared approximately 18 days after larval settlement (Fig. 3B), suggesting that post-larval abalone were removing the top valves of the diatom and thus gaining access to the diatom cell contents.

The growth-rate of the post-larval abalone was relatively constant throughout the experiment with a mean growth-rate of 31.4 µm/ day (S.E. 5.2 µm) 11 days after settlement, 33.3 µm/ day (S.E. 1.4 µm) 18 days after settlement and 33.9 µm/ day (S.E. 0.8 µm) 53 days after settlement.

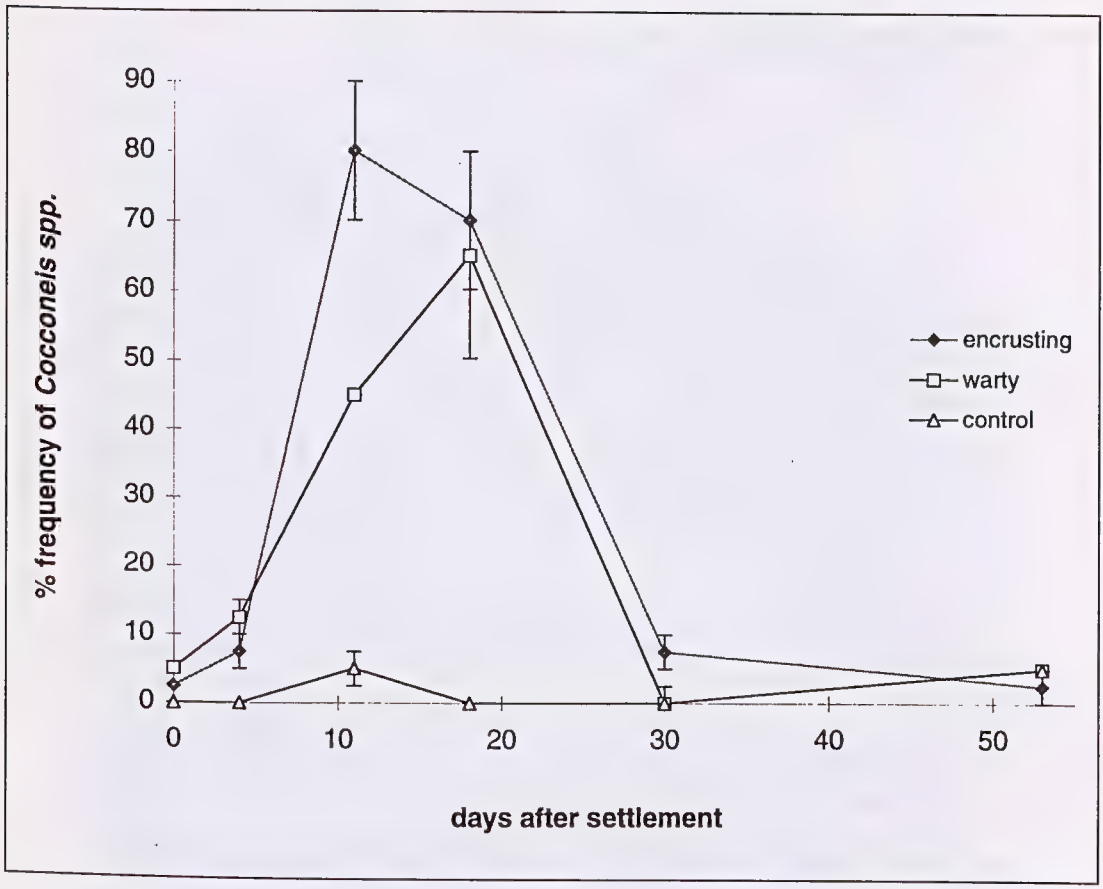

Figure 2. Changes in frequency of occurrence of *Cocconeis* spp. on the surface of *Phymatolithon repandum* when grazed by post-larval abalone (*Haliotis rubra*). Vertical bars indicate the standard error.

Cell sloughing of NCA

Cell sloughing was observed on the control pieces of *P. repandum* and occurred significantly more on the warty than on the encrusting growth-form of the treatment (1-way ANOVA, $p<0.047$). The mean frequency of cell sloughing was 16% (S.E. 4.3%) for the encrusting and 45% (S.E. 12.1%) for the warty growth-form. Cell sloughing was intense both around and between warty protuberances (Fig. 4A). Figure 4B shows an area of intensive sloughing in detail. The outermost cells become detached in sheets of numerous cells and subsequently removes all epiphytes occurring on the surface.

Abalone radula

The post-larval abalone radula has numerous teeth per row (Fig. 5A). Each row consists of 1 median tooth with a smooth edge, 5 (2+3) strong lateral teeth on each side with sharp endings for rasping, and numerous marginal teeth with serrated edges. The marginal teeth are similar to the outermost lateral teeth, but become progressively thinner.

Each tooth has a width-to-length ratio of approximately 1:2. The teeth of *Haliotis rubra* have a clearance angle (angle from the coralline surface to the back of the tooth) of about 8°.

Figure 3. Abalone post-larvae grazing on the surface of *Phymatolithon repandum*. A: Post-larvae 11 days after settlement, *Cocconeis sp.* proliferating on the surface. B: Grazed surface of NCA with bottom, raphe-bearing valve of the diatom *Cocconeis scutellum* still attached to the surface.

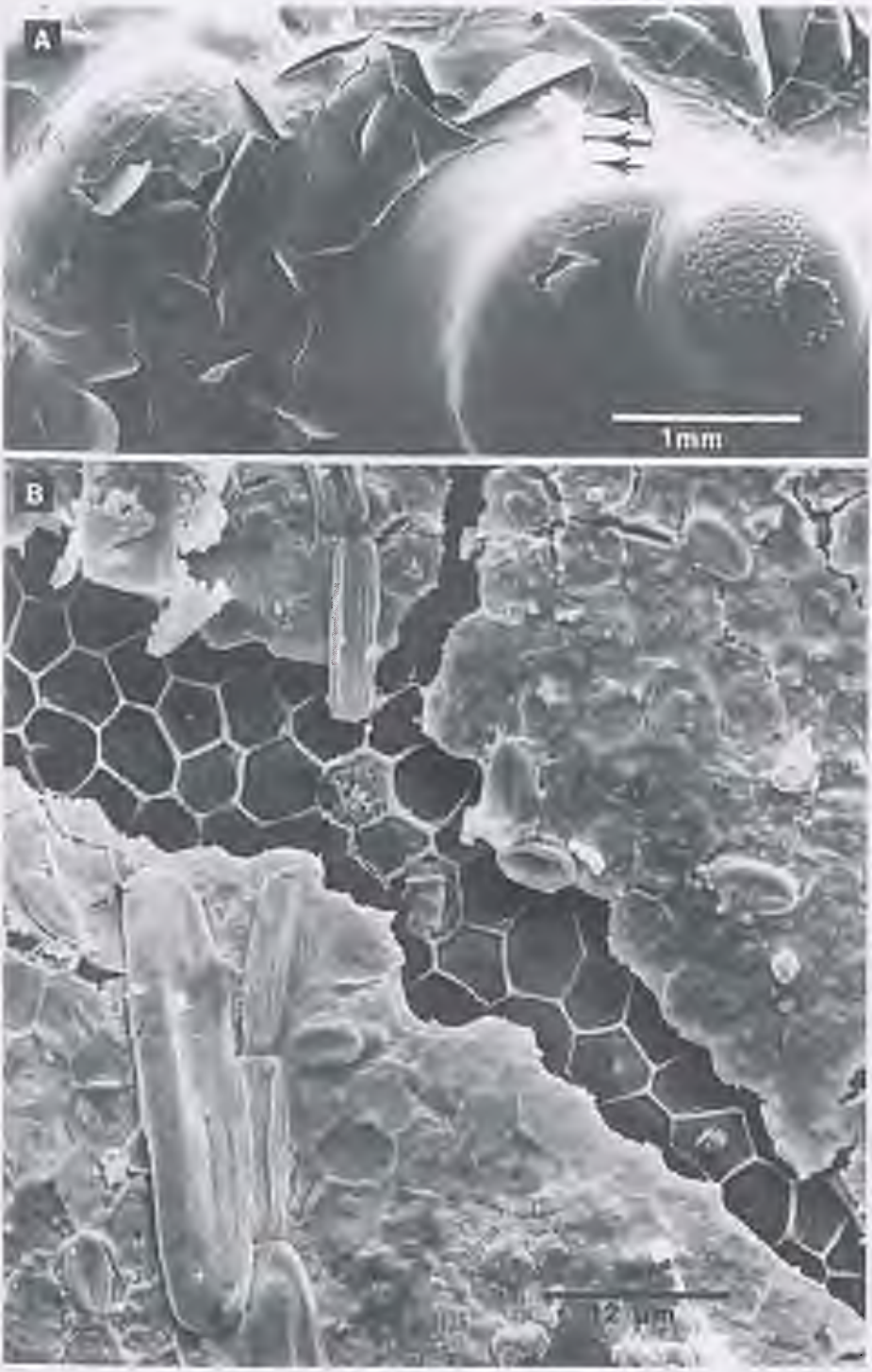

Figure 4. Epithallial cell sloughing of *Phymatolithon repandum*. A. Warty growth-form with intensive cell sloughing (arrows). B. Outermost layer of cells sloughing off and effectively remove diatoms growing on the surface.

Grazing marks matching the pattern of post-larval abalone radulae were not found on the surface of *P. repandum* during the 53 days after settlement. The polysaccharide layer together with the outermost cells of the thallus were often missing on both the grazed and ungrazed (control) pieces.

Discussion

Distribution of the dominant diatoms on *P. repandum*

The low frequency of diatoms observed on freshly sampled NCA is comparable to non-living substrata such as rocks but not other macroalgae (Round *et al.* 1990). Non-living substrata are less favourable to diatom growth than macroalgae and seagrasses and thus support a low frequency of diatoms (Hudson & Bourget 1981).

Eckman and Norwell (1984) noticed that on the bottom of bumps, areas with a specific horseshoe vortex effect of flow, the microbial activity and mucus binding capacity is enhanced. Consequently, we expected a higher frequency of diatom occurrence on the warty growth-form. However, we did not notice any difference between the two growth-forms, neither on freshly sampled pieces or on pieces sampled throughout the experiment. The observed higher frequency of cell sloughing on the warty growth-form (warty treatment and control) could have offset the expected growth-form effect and thus the frequency of diatoms was similar on both growth-forms of *Phymatolithon repandum*.

In this study, species of *Cocconeis* were observed to be the dominant diatoms on the surface of *P. repandum*. In agreement with our findings, Kawamura *et al.* (1992) observed that in areas dominated by crustose coralline algae, diatoms with strong adhesive solitary forms such as *Cocconeis* spp. dominate the benthic diatom population. In addition, Hudon and Bourget (1981) found that *Cocconeis* spp. and *Amphora* spp. were the first and major colonizers on artificial plates from May to mid August (northern hemisphere). They noticed a change in diatom dominance at the beginning of September which suggests that the dominance of *Cocconeis* spp. may be seasonal.

Effect of post-larval grazing

Four to 11 days after larval settlement the populations of *Cocconeis* spp. increased exponentially on both growth-forms of NCA. We did not find any bottom valves of diatoms on the NCA surface at this young age, suggesting that post-larval abalone do not graze on *Cocconeis* spp. at this stage. Species of *Cocconeis* are known to have high adhesive strength (Kawamura *et al.* 1995) and young post-larvae might not be able to detach the valves from the surface of *P. repandum*. In addition, competing diatoms and bacteria may have been preferentially consumed at this early stage. Young post-larvae are known to feed on loosely attached diatoms. The extracellular mucus associated with these diatoms is considered to be a good food source (Kawamura and Takami 1995; Kawamura 1996). The grazing activity and metabolic processes of post-larvae up to 11 days after larval settlement, seems to allow the *Cocconeis* population to proliferate which ensures enough food supply for the abalone in coming weeks. In the control, without abalone grazing activity, the *Cocconeis* population never exceeded a 5% frequency. In agreement, Suzuki *et al.* (1987) and Matthews and Cook (1995) noted that under the grazing pressure of juvenile abalone, prostrate diatoms like *Cocconeis* spp., start to proliferate. After several days artificial plates are covered entirely with these diatoms (Suzuki *et al.* 1987). However, none of them observed post-larvae or juveniles grazing on *Cocconeis* spp.

We measured a mean growth-rate of ca. 30 µm/ day 11 days after settlement which stayed relatively constant throughout the experiment. Garland *et al.* (1985) settled larvae of *Haliotis rubra* onto NCA in 1000–l culture bins. The larvae grew to a mean size of 692± 33 µm in 42 days (10.5 µm/day) which is considerably lower than the growth-rate we calculated. However, they did not have any measurements for the first two weeks of rearing. Kawamura and Takami (1995) measured the mean growth-rate of the post-larval abalone *Haliotis discus hannai*, settled in tissue culture wells with only *Cocconeis scutellum* as a food source. They recorded a growth-rate of 24.7±1.6 µm/day

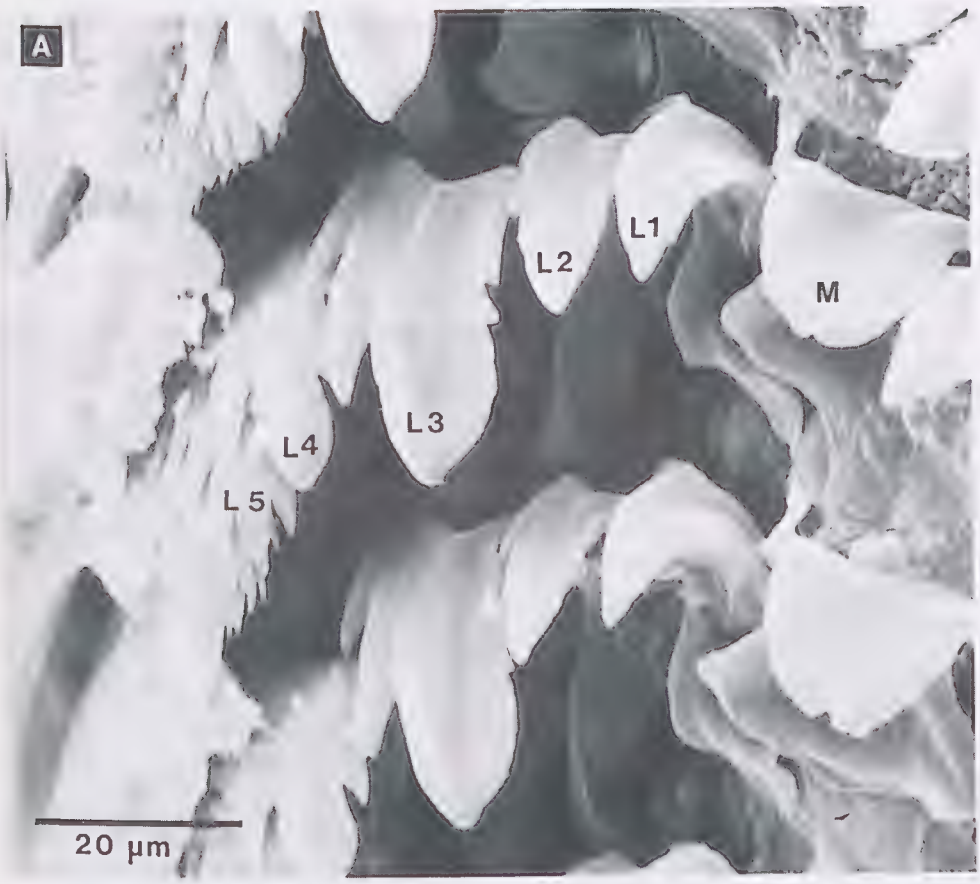

Figure 5. Part of the radula of a post-larval abalone (*Haliotis rubra*) with 1 median (M) and 5 lateral teeth (L1–L5).

from 0 to 10 days after settlement. The growth rate decreased 10 days after settlement (7.5±2.1 µm/day) and was significantly lower than those of abalone fed on other species of diatoms. Takami *et al.* (this issue) settled abalone larvae of the same species onto small rocks encrusted with the NCA *Lithophyllum yessoense*. When treated with GeO_2 to reduce the diatom growth on surfaces of *L. yessoense*, the growth-rate of the abalone did not differ from untreated *L. yessoense* surfaces during the first seven days of rearing. However, the growth-rate of the post-larvae *H. discus hannai* in this experiment was both constant and higher on the surface of *L. yessoense* (treated 37.0 ± 0.7 µm/day and untreated 40.9 ± 0.7 µm/day) compared to Kawamura and Takami's (1995) experiment, in which the larvae were grown on diatoms only. In agreement with our results, this suggests that post-larval abalone must find an additional food source derived from the NCA rather than the diatoms occurring on its surface. This food source is important during the first week of rearing.

Giraud and Cabioch (1976) describe a layer of polysaccharide material covering the external part of the thallus of a variety of different species of NCA. The cells just below that layer have uncalcified cell walls at their external pole and vesicles full of polysaccharide fibrils which can open through the plasmalemma and diffuse into the outermost layer. Garland *et al.* (1985) suggested that post-larval abalone (6 weeks old) graze on the surface mucus, polysaccharide layer and the epithallial cell content of the NCA.

Diatoms with valves which can be ruptured by the abalone radula, such as the valves of *Cocconeis* spp., are considered to be a high value food source for post-larval abalone, approximately 1mm in

shell length (Kawamura 1996). Our findings suggest that post-larvae of *Haliotis rubra* start feeding on diatoms about 18 days after settlement, when they are approximately 800 µm in shell length. In addition, Takami *et al.* (1997b) stated that post-larvae of *H. discus hannai* are able to access diatom cell contents once they reach about 800 µm. Takami *et al.* (this issue) found a higher growth-rate of *Haliotis discus hannai* fed on NCA with diatoms, than on NCA without diatoms, 2 weeks after settlement. In agreement with our findings this also suggests that the NCA alone provides enough food for the post-larvae until 2 weeks after settlement and /or post-larvae can not utilize the diatom food source until approximately 2 weeks after larval settlement.

Cell sloughing of NCA

Cell sloughing was observed on the control pieces and more frequently on the warty than on the encrusting growth-form of *P. repandum*. This difference could explain the lower frequency of diatoms on the warty growth-form in Fig. 2 and the overall low diatom frequency of the ungrazed control. It also suggests that cell sloughing intrinsically controls overgrowth by diatoms and other epiphytes when grazers are absent. Cell sloughing may also facilitate the occurrence of early successional diatom species such as *C. scutellum* (Hudson and Bourget 1981) by providing new, uncolonised substrata.

The polysaccharide layer together with epithallial cells of the coralline thallus were partially missing on all pieces observed. Garland *et al.* (1985) report that post-larvae (6 and 13 weeks old) of *Haliotis rubra* remove and ingest the polysaccharide layer and epithallial cells of NCA. In this study grazers in the field (before the NCA had been sampled) or the post-larval abalone (up to 53 days after settlement) might have removed the outermost cells and polysaccharide layer without leaving any obvious, deeply penetrating grazing marks.

Abalone radula

Abalone have a rhiphidoglossan radula like many primitive herbivorous prosobranchs (Crofts 1929). The teeth of post-larval abalone have a low clearance angle. A clearance angle close to 0° results in a tooth sliding across the surface rather than cutting into the surface of the algae (Padilla 1985). This might explain why we did not observe grazing marks of post-larval abalone on the surfaces of *P. repandum.* Given the low clearance angle of the teeth, it seems that abalone post-larvae would be more efficient grazers on flat surfaces *eg.* encrusting compared to a warty growth-form of NCA.

We concluded that abalone post-larvae must find an additional food source derived from the NCA before grazing on the diatoms, because of the high growth-rate during the first and second week of rearing and the constancy throughout the experiment.

Both mechanisms, the cell sloughing of *P. repandum* and grazing by post-larvae, seem to enhance the quality of the diatom assemblage on NCA as a food source for post-larval abalone. Cell sloughing controls overgrowth by diatoms and other epiphytes (Keats *et al.* 1997) which is known to be advantageous during the early life history of abalone (Shepherd & Daume 1996). Grazing by post-larval abalone seems to encourage proliferation of *Cocconeis* spp. which provides a good food source for the coming weeks.

Further experiments are required to gain a better understanding of the interactions of both mechanisms on epiphytic diatom assemblages and their effect on abalone settlement and early growth.

Acknowledgments

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Variable response from abalone larvae (*Haliotis iris, H. virginea*) to a range of settlement cues

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Abstract

We exposed competent larvae of two abalone species (*Haliotis iris* and *H. virginea*) to a range of potential settlement cues, and observed a hierarchy of responses. The end point and the timing of the settlement process varied, depending on the cue provided. Response was qualitatively consistent between experiments, but showed some quantitative variation. Rocks coated with crustose coralline algae (CCA) induced 80–100% of larvae to attach and metamorphose, and 50–100% to grow peristomal shell, within 2 days. A distilled water extract of CCA induced almost 100% attachment within 2 days, but metamorphosis occurred gradually between 2 and 5 days. Shell growth was minimal, apparently inhibited by chemical interference from the extract. γ-Aminobutyric acid (GABA, 1 μM) induced 90–100% attachment and 20–60% metamorphosis in *H. iris* within 2 days. For *H. virginea,* 1 μM GABA induced attachment (65–100%) but typically only 0–5% metamorphosis within 2 days. KCl (10 mM) added to seawater induced attachment (50–70%) but less than 10% metamorphosis. Different diatoms induced responses ranging from rapid or gradual induction of metamorphosis, to minimal response. Some combinations of cues produced synergistic effects. *E.g.*, GABA (1 μM) plus diatom biofilm induced more metamorphosis (49%) than biofilm (28%) or GABA (1%) alone. Addition of CCA crude extract to the same biofilm hastened metamorphosis. Observations of attachment without subsequent metamorphosis suggest that there may be separate cues for attachment and metamorphosis. This study highlights the complexity of the settlement response in abalone.

Introduction

Most marine invertebrates have a free swimming larval stage prior to a benthic adult phase. For many species, including abalone, the transition from swimming larva to crawling post-larva (herein referred to as "settlement") requires a chemical cue (Crisp 1974; Pawlik 1992). Larval abalone settle and metamorphose in response to various substances including intact crustose coralline algae (CCA), extracts from CCA (Morse *et al.* 1980), γ-aminobutyric acid (GABA – Morse 1984; Yang and Wu 1994), excess potassium ion (Yool *et al.* 1986; Yang and Wu 1995) and cultures of benthic diatoms (Kawamura and Kikuchi 1992).

Some variations in the settlement response of abalone have been documented, *e.g.* Searcy-Bernal *et al.* (1992) found that a diatom-based biofilm induced metamorphosis more slowly than 1 μM GABA or mucus treatments; and Morse (1984) reported that concentrations of GABA exceeding 1 μM induced attachment, but inhibited metamorphosis. However, there has been little detailed comparison of settlement responses across a range of different cues. Such a comparison needs to account for variation between batches of larvae (Dineen and Hines 1994) and differences in methods between

experiments (Morse 1992). One aim of this study was to determine the response of a single batch of abalone larvae to several cues which have previously been shown to induce settlement in other abalone (see above). Another was to quantify the extent of inter-experiment variability (using standardised methods) for a subset of the cues.

Variation in the timing or completeness of the settlement response is important in abalone culture. Mortality during the first two months is typically 85–100% (Searcy-Bernal *et al.* 1992), and failure to metamorphose can be an important factor (Ebert and Houk 1989). The speed of the settlement response, the proportion of larvae responding, and the distinction between attachment and metamorphosis are all important in managing the settlement process in commercial hatcheries.

Anecdotal reports of larvae of *H. australis* Gmelin 1791 attaching, but later resuming swimming (G. Moss, pers. comm.), suggest that attachment and metamorphosis may be uncoupled, and triggered by different cues. By quantifying the components of the settlement response separately over time, we hoped to gain insights into the cue(s) for attachment, metamorphosis and post-larval development.

This paper describes the timing and end point of the settlement response to a range of cues, using two New Zealand abalone species – *Haliotis virginea virginea* Gmelin 1791 and *H. iris* Gmelin 1791.

Materials and methods

Larval rearing

Spawning was induced by adding hydrogen peroxide and sodium hydroxide (final concentrations of 6 mM and 1 mM respectively) to the seawater bathing ripe adults (Morse *et al.* 1977). Larvae were hatched and reared in 1 µm filtered, flowing, natural seawater. The rearing system was cleaned, and the filters were changed every 2 days. *H. virginea* were reared at the Glenhaven Aquaculture Centre Ltd in Nelson. *H. iris* larvae were initially reared at Rainbow Abalone Ltd in Taranaki, or the National Institute of Water and Atmospheric Research in Wellington. Operculate larvae were transported to Nelson using established techniques (Tong and Moss, 1992) then reared at the Glenhaven Aquaculture Centre until used in settlement bioassays. Larval stage was determined by examining radula development (Tong and Moss 1992). Larvae gained competence to settle at ca. ~100°C.days of age, which corresponded to five rows of fully chitinized radula teeth (Roberts, unpubl. for *H. virginea*; Moss and Tong (1992) for *H. iris*).

Settlement cues

Pebbles coated with CCA (>90% cover of "encrusting" to "warty" growth form (Shepherd and Daume 1996) species unknown) were collected from Cable Bay, Nelson (173°24'E, 41°11'S) and held in flowing raw sea water until assayed. Crude extract of CCA was prepared by soaking CCA-coated rocks in distilled water for 36 hours. Extract (unfiltered) was evaporated to dryness at 35°C, resuspended in distilled water and stored at –20°C. Extract was assayed at 0.44 mg dry extract per ml of assay medium for *H. virginea*, but at 0.22 mg/ml for *H. iris* because the higher dose was mildly toxic. A partially purified extract was prepared by anion exchange chromatography. Crude extract was buffered at pH 6.0 with 20 mM Bis-Tris (Sigma), filtered (0.45µm, Millipore HAWP) then pumped through a column of Q-Sepharose Fast Flow (Pharmacia, 12 cm x 2.5 cm diam.) previously equilibrated with 20 mM Bis-Tris, pH 6.0. Bound material was eluted with 2.0 M NaCl, then bioassayed as described below. Active fractions were pooled, and stored at –20°C. This pooled sample is referred to as "partially purified extract" below.

Potassium chloride (KCl, 10 mM, Baker) was made up in 1 µm filtered, UV-treated natural seawater ("FSW"). A frozen stock solution of 10 µM GABA (Sigma) in distilled water was diluted with FSW to a final concentration of 1 µM. The salinity change caused by the distilled water did not affect the larvae. FSW was run as a negative control.

Benthic diatoms were isolated from Nelson coastal waters (*Nitzschia* sp. 1, *Nitzschia* sp. 2, *Navicula* sp. 1) or obtained from the Tohoku National Fisheries Research Institute in Japan (*Cocconeis scutellum* var. *parva* Cleve 1895). Diatoms were cultured in F/2 medium (Guillard 1975) in tissue culture dishes (Falcon 3043) on a 12:12 LD cycle at 30–70 $\mu E.m^{-2}.sec^{-1}$. The diatoms and associated microbes formed a film on the floor and walls of the chambers. Diatom cell density was determined by replicate counts of the chamber floor immediately prior to assays. Cultures were not axenic. For settlement bioassays, F/2 growth medium was replaced with FSW, and larvae were added to the culture chamber.

Larval settlement bioassays

For settlement bioassays, competent larvae were transferred to 12 well tissue culture dishes (Falcon 3043) at densities of ca. 50–100 per well. Assays were run in FSW containing 150 µg/ml each of Penicillin G sodium (Biochemie) and Streptomycin sulphate BP (Sigma), total assay volume 3.37 ml. These antibiotics ensured virtually nil mortality of larvae/post-larvae. Assay dishes were wrapped in foil and incubated at 17 ± 1°C for up to 12 days without water change.

Larval settlement was quantified by inspecting the floor and walls of the assay chamber, and the surface film of the assay water, with inverted and dissecting microscopes. Swimming larvae could not be accurately counted due to their mobility. Where necessary, larvae were killed and counted at the end of the experiment to allow calculation of the number of swimming larvae by subtracting the sum of all non-swimming larvae (at day 2, 4 etc) from the total number of larvae. Abalone attached to pebbles coated with CCA were dislodged with a water jet to allow accurate counting. We calculated the percentage of live animals showing: (a) "shell growth" (velum shed, peristomal shell visible); (b) "metamorphosis" (velum shed, with or without peristomal shell growth); (c) "attachment" (metamorphosed or attached by foot).

Table 1 details the aims of this study, and the larvae and treatments used.

Table 1. Study aims, with details of larvae and treatments used. Number of rows of teeth on radula is a measure of larval development at the time the experiment was set up. Larvae with >5 rows of teeth are competent to settle. n = number of replicate treatments (50–100 larvae per replicate). CCA = crustose coralline algae; FSW = 1 µm filtered, UV-treated, natural seawater.

Aim	Abalone species	Spawning date	Rows of teeth on radula	n	Treatments
Determine response of a single larval batch to a range of settlement cues, and examine differences between the two abalone species	*H. iris* *H. virginea*	12/9/96 5/9/96	10 6	6 5	Intact CCA, CCA extract, GABA (1 µM), KCl (10 mM), FSW control
Determine whether chemical interference from CCA extracts inhibits post-larval shell growth	*H. virginea*	15/7/96	10	Various – see Table 2	Partially purified CCA extract
Demonstrate the range of settlement responses elicited by different diatom-based microbial films	*H. iris* *H. virginea*	12/9/96 1/8/96	10 9	6 4	Cultures of four benthic diatom strains (not axenic)
Examine the potential for enhancing settlement by applying combinations of cues	*H. virginea*	1/8/96	9	4	GABA + *Nitzschia* sp. 1, CCA extract + *Nitzschia* sp. 1
Quantify variation in settlement response between experiments for selected cues	*H. iris* *H. virginea*	Various – see Table 3	7–12 – see Table 3	2–5 – see Table 3	Intact CCA, GABA (1 µM), FSW control

Results

Response to different settlement cues

Complete and rapid settlement was induced by CCA-coated pebbles for both *H. iris* and *H. virginea* (Figure 1a–b). Post-larvae on CCA-coated pebbles fed actively and produced faecal material. In contrast, when larvae were incubated without any cue, less than 10% of larvae attached, and virtually none metamorphosed (Figure 1, i–j).

The response to a distilled water extract of CCA differed in two respects from the response to intact CCA. Firstly, while the extract induced attachment and metamorphosis, it did not induce shell growth (Figure 1c–d). Secondly, metamorphosis was delayed relative to CCA, and occurred largely between 2 and 5 days after introduction of the extract (Figure 1c–d). In extract-induced post-larvae, the mantle margin migrated to the edge of the larval shell, and feeding commenced, but was weak and intermittent. The post-larvae produced no faecal material, and no more than a short stub of peristomal shell. Two lines of evidence suggest that chemical interferences were responsible for the lack of shell growth. (1) When larvae were added to *Nitzschia* sp. 1 with and without CCA extract, mean length of new shell was 3–4 times lower in the presence of crude extract (Table 2, t=4.5, p<0.001). (2) When larvae were incubated with partially purified extract at near its lowest effective dose, significantly more post-larval shell was added than at a four-fold higher concentration (Table 2, t=10.1, p<0.001).

Testing of pure compounds revealed further differences in larval responses. *H. iris* exposed to 1 µM GABA showed high initial attachment (which fell within several days) and moderate percentage metamorphosis (Figure 1e). The resulting post-larvae displayed normal feeding movements despite a virtual absence of particulate food, but did not produce faecal material. Yolk was still visible in post-larvae 10 days after introduction of the GABA. Post-larvae grew 30–120 µm (n=46) of peristomal shell length during 10 days incubation, with most of the growth occurring in the first 4 days. For *H. virginea* the response was different – 1 µM GABA induced attachment but no metamorphosis (Figure 1f).

Potassium chloride (10 mM added to seawater) induced moderate attachment, but very little metamorphosis of either abalone species, even after 11 days of exposure (Figure 1g–h). The percent attachment fell after several days of incubation (Figure 1g–h).

With both KCl and GABA we have tried many variations on our methods (assay chambers up to 400 ml, no antibiotics, different antibiotics, range of KCl/GABA concentrations), but have always obtained results similar to those presented.

Table 2. Comparison of mean (± SE) width of post-larval shell produced by *H. virginea*: (a) on *Nitzschia* sp. 1 (1.1 ± 0.1 x 10^5 diatom cells.cm^{-2}, not axenic) in presence/absence of CCA extract, or (b) in high versus low doses of partially purified CCA extract. Post-larval shell growth was measured as the maximum width of the peristomal shell band. n = number of abalone measured.

Treatment	Post larval shell width (µm)	Days of incubation	n
a) Diatom with/without CCA extract			
Nitzschia sp. 1 with CCA extract (0.44 mg/ml)	16 ± 3.9	10	31
Nitzschia sp. 1 without CCA extract	56 ± 7.9	10	39
b) Partially purified CCA extract			
68µL – high dose	6.0 ± 1.6	6	31
17µL – low dose	26.1 ± 1.2	6	31

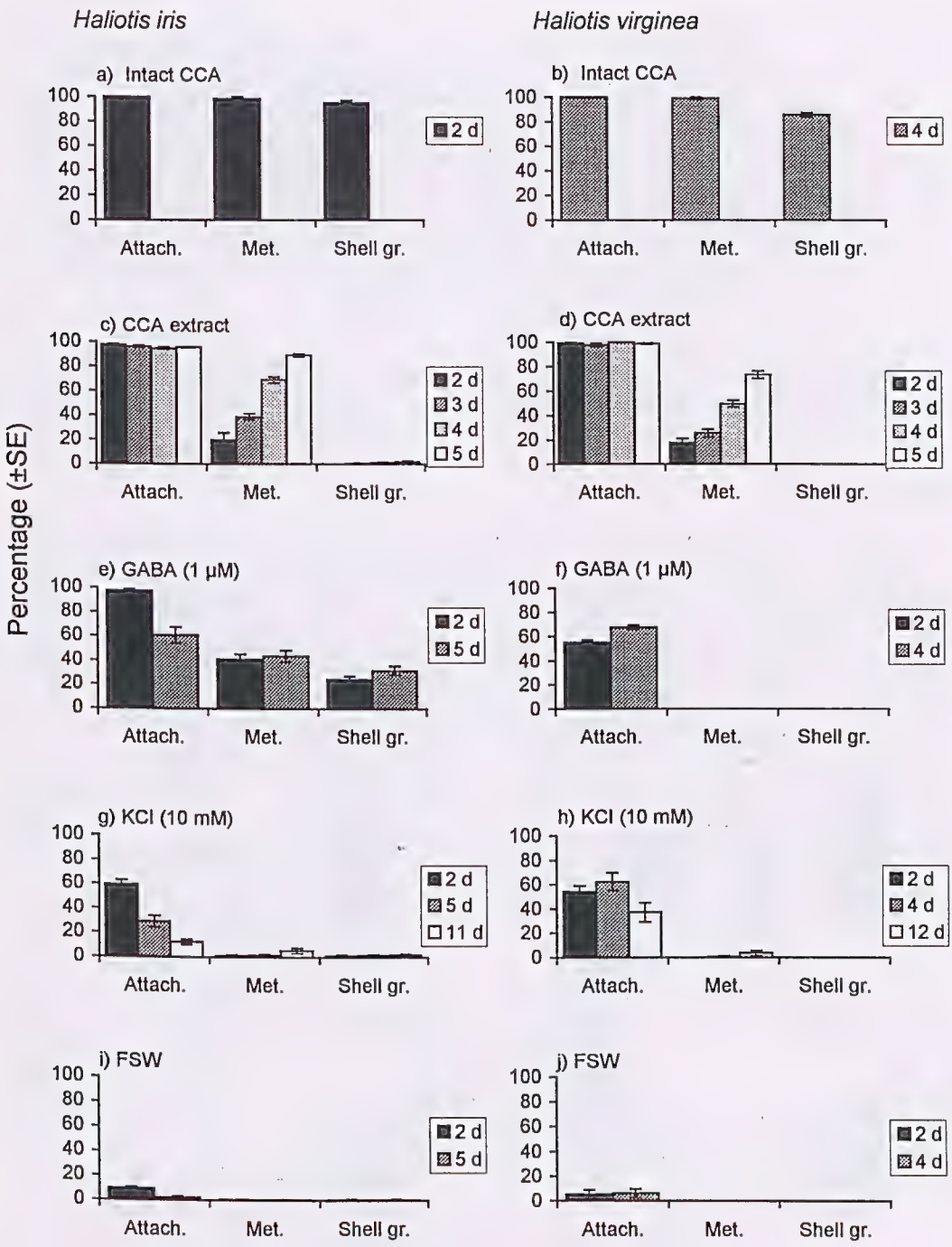

Figure 1. Comparison of the settlement response of abalone larvae (single batch for each species) to four settlement cues and a negative control (FSW). Intact CCA = pebbles covered with crustose coralline algae; CCA extract = distilled water extract of CCA (0.44 mg/ml); FSW = 1 µm filtered, UV-treated, natural seawater. Attach. = attachment, Met. = metamorphosis, Shell gr. = shell growth. Legend gives number of days of incubation at time of counting. *H. iris*: n=6. *H. virginea*: n=5.

Responses to diatom films

Data from four diatom strains (not axenic) are presented to demonstrate that microbial films can elicit the full range of responses described above for other cues. *Nitzschia* sp. 1 induced rapid attachment, metamorphosis and shell growth in *H. iris* (Figure 2a) with effectiveness comparable to intact CCA. *Cocconeis scutellum* var. *parva* also induced all three components of settlement in *H. virginea*, but the onset of metamorphosis and shell growth was gradual, occurring largely after 5–10 days of exposure (Figure 2b). *Navicula* sp. 1 induced moderate percent attachment, but virtually no metamorphosis in *H. virginea* (Figure 2c), echoing the response of this species to 10 mM potassium chloride and 1 µM GABA. *Nitzschia* sp. 2 was a very weak inducer of attachment and metamorphosis in *H. iris* (Figure 2d). Post-larvae which metamorphosed on diatom films showed normal feeding behaviour and produced faecal material.

Synergistic effects between cues

In experiments examining the enhancement of settlement responses by combining stimuli, we have observed some synergistic interactions between pairs of cues, *e.g.* GABA (1 µM) combined with *Nitzschia* sp. 1 induced more metamorphosis in *H. virginea* than the sum of the two cues alone (Figure 3). Addition of CCA extract to a *Nitzschia* sp. 1 culture hastened metamorphosis of *H. virginea* (Figure 4).

Inter-experiment variability in response

In order to examine inter-experiment variability, we compared the settlement response over a series of experiments using standard procedures. Data are presented for three treatments representing nil (FSW), partial (1 µM GABA) and complete (intact CCA) settlement responses. Detailed data (Table 3) are presented for *H. virginea* only, except for GABA, where the response from the two species differed.

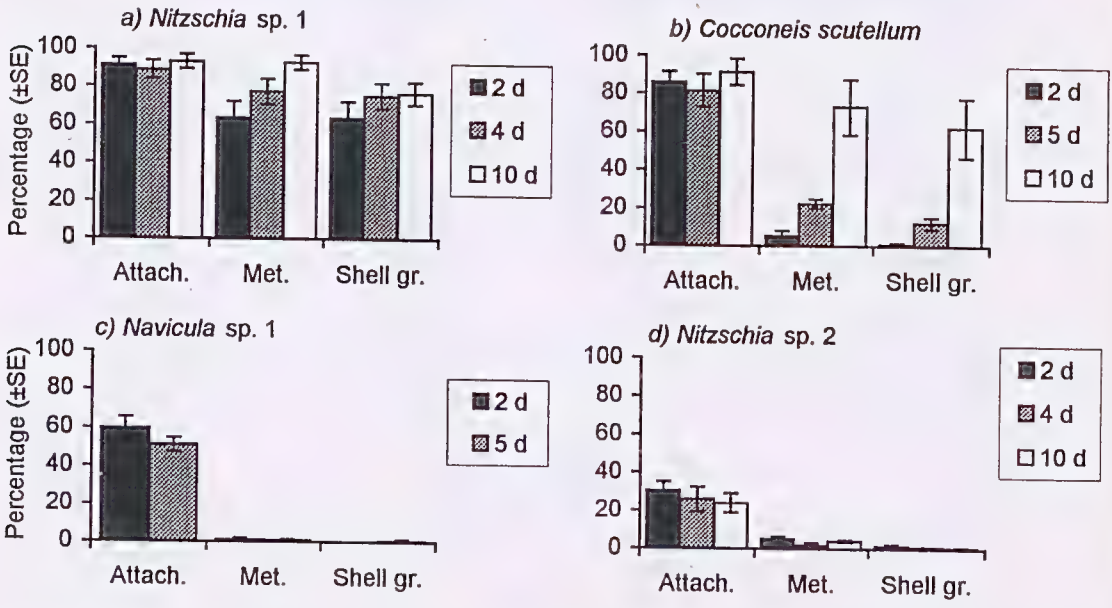

Figure 2. Settlement response of abalone larvae to cultures of selected benthic diatoms, illustrating the range of responses possible. Cultures were not axenic. Graphs a) and d) are for *H.iris* (n=6); b) and c) are for *H. virginea* (n=4). Mean (± SE) diatom densities were as follows: a) 4.0 ± 0.3 x 10^5.cm^{-2}; b) 8.5 ± 0.6 x 10^3.cm^{-2}; c) 1.6 ± 0.1 x 10^5.cm^{-2}; d) 1.4 ± 0.1 x 10^5.cm^{-2}. Attach. = attachment, Met. = metamorphosis, Shell gr. = shell growth. Legend gives number of days of incubation at time of counting.

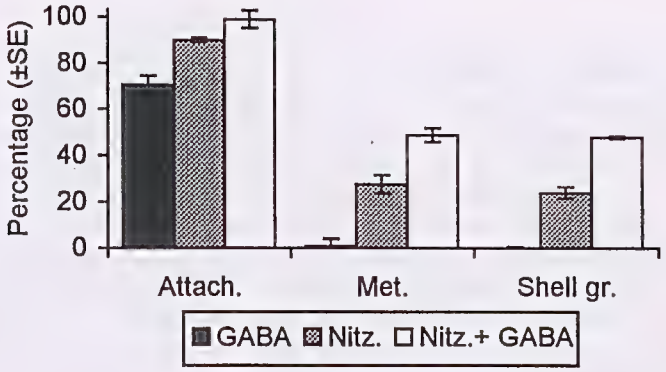

Figure 3. Settlement response of *H. virginea* to combined settlement cues. Combination of GABA (1 μM) plus *Nitzschia* sp. 1 ($1.1 \pm 0.1 \times 10^5$ diatom cells.cm^{-2}, not axenic) produced a synergistic effect which increased percent metamorphosis and shell growth of *H. virginea* after 5 days. Attach. = attachment, Met. = metamorphosis, Shell gr. = shell growth. n = 4.

The larval settlement response to CCA was consistent over multiple experiments with *H. virginea*, although percentage shell growth showed moderate variability. The response to CCA was also consistent for *H. iris* – two previous experiments produced 98–100% attachment and metamorphosis after 2 days of incubation (data not shown).

The larval response to GABA was qualitatively consistent, but showed some quantitative variation. In particular, metamorphosis of *H. iris* and attachment of *H. virginea* varied between experiments (Table 3). The variation in settlement response was not related to larval development (as indicated by rows of radula teeth in Table 3). Percent attachment of *H. iris* was always high, and did not parallel variations in metamorphosis. The distinction between the response of the two abalone species to GABA (i.e., *H. virginea* attached but failed to metamorphose) was maintained across multiple experiments.

FSW was a valid negative control, producing consistently low attachment and virtually no metamorphosis or shell growth.

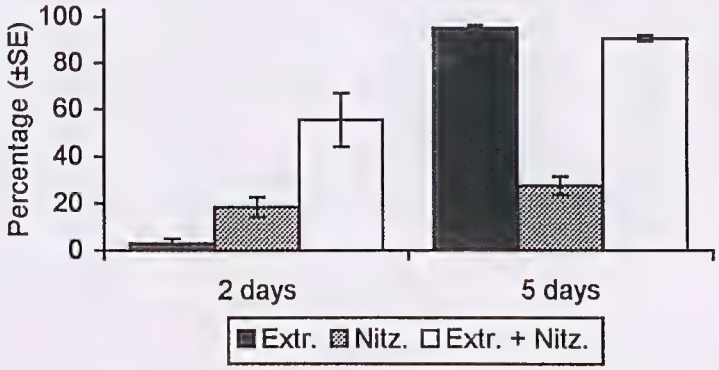

Figure 4. Metamorphosis of *H. virginea* in response to combined settlement cues. Combination of a crustose coralline algae extract ("Extr.", 0.44 mg/ml) and *Nitzschia* sp. 1 ("Nitz.", $1.1 \pm 0.1 \times 10^5$ diatom cells.cm^{-2}, not axenic) produced a synergistic effect which accelerated metamorphosis of *H. virginea*. X-axis labels show number of days of incubation at time of counting. n = 4.

Discussion

Complex response to range of settlement cues

The results demonstrate the complexity of the settlement responses of *H. virginea* and *H. iris*. Complete and rapid metamorphosis was induced only by CCA-coated pebbles and one of the diatom strains tested. The response to all other cues tested varied in either the timing or end-point of the response, or in the proportion of the larvae responding.

Table 3. Comparison of settlement response to selected cues from repeated experiments using standard procedures. Data are mean ± SE, counted after 2 days and 4 days of incubation. "Rows teeth" is the number of chitinized rows of teeth on the radula when the experiment was set up. nd = not determined. n = number of replicate treatments (50–100 larvae per replicate). CCA = crustose coralline algae-covered pebbles; FSW = 1 µm filtered, UV treated natural seawater. Attach. = attachment, Met. = metamorphosis, Shell gr. = shell growth.

		2 day counts			4 day counts			
Spawning date	Rows teeth	% Attach.	% Met.	% Shell gr.	% Attach.	% Met.	% Shell gr.	n
CCA – *H. virginea*								
15/7/96	9	95 ± 2.4	89 ± 3.7	74 ± 4.0	nd	nd	nd	5
29/5/96	10	95 ± 0.7	91 ± 1.6	81 ± 9.6	nd	nd	nd	3
17/4/96	10	98 ± 1.0	96 ± 0.4	87 ± 5.1	nd	nd	nd	2
6/5/95	7	98 ± 1.0	95 ± 0.9	58 ± 15.5	nd	nd	nd	3
27/4/95	8	96 ± 1.0	92 ± 4.4	71 ± 15.4	nd	nd	nd	3
27/10/94	7	83 ± 3.2	78 ± 3.9	49 ± 9.7	nd	nd	nd	3
4/10/94	7	100 ± 0.3	97 ± 1.1	50 ± 9.4	nd	nd	nd	5
Mean±SE		95 ± 2.1	91 ± 2.4	67 ± 5.7				
1 µM GABA – *H. virginea*								
15/7/96	9	65 ± 10.5	3 ± 1.9	0 ± 0	nd	nd	nd	5
29/5/96	10	64 ± 4.2	0 ± 0	0 ± 0	nd	nd	nd	3
17/4/96	10	71 ± 3.2	0.9 ± 0.9	0 ± 0	55 ± 7.2	1.0 ± 0.9	0 ± 0	2
6/5/95	7	88 ± 2.3	0 ± 0	0 ± 0	92 ± 1.0	0.5 ± 0.5	0 ± 0	3
27/4/95	8	81 ± 1.8	0 ± 0	0 ± 0	93 ± 1.7	0.4 ± 0.4	0.4 ± 0.4	3
27/10/94	7	65 ± 6.4	0 ± 0	0 ± 0	65 ± 8.9	1.4 ± 1.4	0 ± 0	3
4/10/94	7	96 ± 1.4	17 ± 2.2	0 ± 0	95 ± 1.7	39 ± 4.5	0.4 ± 0.4	5
Mean±SE		76 ± 4.8	3 ± 2.4	0 ± 0	80 ± 8.3	8 ± 7.6	0.2 ± 0.1	
FSW – *H. virginea*								
15/7/96	9	1.4 ± 0.2	0.3 ± 0.2	0 ± 0	nd	nd	nd	5
29/5/96	10	8.6 ± 3.3	0 ± 0	0 ± 0	nd	nd	nd	3
17/4/96	10	0 ± 0	0 ± 0	0 ± 0	1.1 ± 1.1	0 ± 0	0 ± 0	2
6/5/95	7	12 ± 0.5	0 ± 0	0 ± 0	19 ± 7.1	0 ± 0	0 ± 0	3
27/4/95	8	14 ± 2.6	0 ± 0	0 ± 0	9 ± 4.4	0 ± 0	0 ± 0	3
27/10/94	7	13 ± 2.1	0 ± 0	0 ± 0	8 ± 4.1	0 ± 0	0 ± 0	3
4/10/94	7	17 ± 3.3	0.3 ± 0.3	0 ± 0	9 ± 4.4	0 ± 0	0 ± 0	5
Mean±SE		9 ± 2.5	0.1 ± 0.1	0 ± 0	9 ± 2.8	0 ± 0	0 ± 0	
1 µM GABA – *H. iris*								
14/08/96	9	96 ± 0.8	36 ± 1.9	31 ± 0.5	93 ± 1.2	90 ± 1.9	84 ± 1.4	2
8/11/95	8	98 ± 0.8	20 ± 2.5	8 ± 2.2	87 ± 3.7	39 ± 4.8	33 ± 4.0	5
8/11/95	12	96 ± 0.7	44 ± 8.1	16 ± 2.7	90 ± 2.1	58 ± 5.7	37 ± 8.8	4
15/09/94	9	92 ± 2.3	61 ± 2.9	7 ± 2.2	nd	nd	nd	5
21/10/93	9	98 ± 2.4	60 ± 1.6	40 ± 4.3	84 ± 0.6	71 ± 6.5	56 ± 1.5	2
Mean±SE		96 ± 1.1	44 ± 7.7	20 ± 6.5	89 ± 1.9	65 ± 10.7	53 ±11.6	

The complexity of the settlement response in *H. iris* and *H. virginea* contrasts with the relatively clear cut results obtained during extensive studies with *H. rufescens* Swainson 1822 by Daniel Morse's group. Under very similar experimental conditions to ours, Morse's group found that larval settlement was either complete and rapid, or absent (*e.g.*, Morse and Morse 1984). In contrast, hatchery studies with *H. rufescens* have produced some conflicting findings (Searcy-Bernal *et al.* 1992; Slattery 1992) and studies of other marine invertebrates (Rodriguez *et al.* 1993) include examples of intermediate settlement responses, such as we describe here.

Complete and rapid settlement in response to intact coralline algae is common to *H. rufescens* (Morse and Morse 1984), *H. iris*, *H. virginea* (this study), and many other marine invertebrates (Rodriguez *et al.* 1993). Beyond this commonality, some differences in settlement behaviour among the three abalone species are evident. *H. iris* and *H. virginea* showed very similar settlement responses (except to GABA – Figure 1), but they differed in several ways from *H. rufescens*.

In *H. rufescens* plantigrade attachment was described as "an accurate indicator of metamorphic commitment" (Yool *et al.* 1986: 258). In conditions equivalent to our FSW negative control, 99–100% of *H. rufescens* larvae continued to swim (Morse and Morse 1984). By contrast, for *H. iris* and *H. virginea*, some cues induced attachment without subsequent metamorphosis (Figure 1f–h, Figure 2b) and our FSW controls typically had 10–15% attachment but virtually no metamorphosis. Attachment was reversible in *H. iris* and *H. virginea*, as seen in GABA, KCl and FSW treatments (Figure 1).

Toxicity of CCA extracts to abalone larvae was reported by Morse and Morse (1984). They found that non-toxic doses of the same extract induced metamorphosis and peristomal shell growth within 36 hours. In contrast, we observed delayed and gradual metamorphosis of *H. virginea* and *H. iris* in response to CCA extracts (Figure 1), and observed minimal peristomal shell growth in the presence of extracts (Table 2). This effect was reduced with lower doses of partially purified extract, suggesting that fully purified inducer compound(s) would not interfere with post-larval development.

Neither *H. iris* nor *H. virginea* responded to 1 μM GABA as convincingly as *H. rufescens* (Morse 1984). There was also a clear distinction between the response of *H. iris* and *H. virginea* to 1 μM GABA, with *H. virginea* showing attachment but not metamorphosis. Morphologically incomplete settlement in response to GABA has been noted for some other marine invertebrate larvae (Rodriguez *et al.* 1993). Excess KCl induced a similar "attachment-only" response for *H. iris* and *H. virginea* in the present study. This is surprising given that many marine invertebrates metamorphose when exposed to this cue (Rodriguez *et al.* 1993). Variations of our bioassay chamber type and antibiotic treatments (to more closely match previous studies) did not alter our results for KCl or GABA.

Our results were in general agreement with previous data for *H. iris*. Moss and Tong (1992) showed strong settlement of *H. iris* larvae on CCA-coated rocks. However, their inferred settlement rate for *H. iris* in response to 1 μM GABA, "diatom film", and "GABA + diatom film" (means of 26%, 45% and 49% respectively after 48 h) were considerably lower than in the present study.

Our results showed a wide range of responses to different diatom cultures. Kawamura and Kikuchi (1992) reported a similar range of responses to diatom cultures with *H. discus hannai* Ino 1953. In contrast, Morse's group found no settlement of *H. rufescens* larvae in response to diatoms or other microbial films (Morse *et al.* 1980) although diatom films are used successfully in hatcheries to induce settlement of *H. rufescens* and many other abalone species (Searcy-Bernal *et al.* 1992).

Source of chemical inducers

Our results show that *H. iris* and *H. virginea* can undergo rapid and complete metamorphosis in response to certain diatom cultures (Figure 2a), so there is no absolute requirement for a CCA-associated inducer. The inducing activity of the diatom culture could derive from diatoms and/or associated microbes such as bacteria. Johnson and Sutton (1994) demonstrated that bacteria mediate the induction of starfish larval settlement on CCA. Since both the CCA and the CCA extracts tested in the present study included their biofilm, our results do not clarify whether the inducer(s) originate from the CCA or associated microflora.

Variability among experiments

The response to a given cue was qualitatively consistent across multiple experiments, but there was some variation in the proportion of the larvae responding. This variation was markedly greater for GABA, than for FSW or CCA (Table 3). The variability would not give rise to false positives or negatives regarding settlement inducers, but could influence the detection of differences in settlement rates between treatments. Such inter-experiment variability may arise from subtle differences between batches of larvae (either from parental influence or environmental conditions during rearing) or through unintentional variations in treatments.

Implications for abalone culture

The complex and varied settlement response documented in this study has implications for abalone culture. Larvae can not be assumed to have metamorphosed when they are no longer swimming. Some settlement surfaces may trigger attachment only, or may require several days to induce high percentage metamorphosis. Fast-acting cues would allow water flow and aeration to be restored promptly to maintain high water quality. There appears to be potential to increase and accelerate settlement by using combinations of cues (Figures 3, 4).

The "attachment-only" cues (Figure 1f–h, Figure 2c) may have application in abalone reseeding programmes based on larval release (e.g., Tong *et al.* 1987). Larvae will be released in areas of suitable habitat to optimise settlement and post-larval survival. Protracted swimming could lead to larvae being advected away from the chosen site to less suitable habitats. If the larvae were primed with an attachment inducer then they would be more likely to settle rapidly to the seafloor, where CCA would trigger metamorphosis.

Separate cues for attachment and metamorphosis?

The fact that attachment can be induced without metamorphosis shows that these events can be uncoupled, and suggests that metamorphosis may require a cue which is separate from, or additional to, the cue for attachment. This possibility has been raised for other marine invertebrates (Hadfield and Pennington 1990; Mokady *et al.* 1992) but has received little attention. Identification of settlement-inducing chemicals from the CCA-biofilm complex (Morse 1992) would help clarify the nature, and number, of settlement cues.

Acknowledgments

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Contribution of diatoms as food sources for post-larval abalone *Haliotis discus hannai* on a crustose coralline alga

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Abstract

Survival and growth rates of post-larval abalone *Haliotis discus hannai* reared on a crustose (non-geniculate) coralline alga (CCA) *Lithophyllum yessoense* with diatoms (CCA + diatoms) and without diatoms (CCA – diatoms) were compared in the laboratory in order to determine the contribution of diatoms on CCA as food sources for post-larvae and the dietary value of CCA themselves. Experiments were performed for 5 weeks with two groups of different developmental stage, newly metamorphosed stage (younger post-larvae) and over 1 mm stage (older post-larvae). Both stages of post-larvae reared on CCA + diatoms grew well (53.4 ± 2.4 μm/day; mean ± SE and 85.0 ± 4.0 μm/day; mean ± SE, respectively). Younger post-larvae grew to over 2 mm in 5 weeks, and older ones reached over 4 mm in 5 weeks. There was no difference in shell length of younger post-larvae reared on CCA + diatom and CCA – diatom at the week 1. However, the mean growth rate of abalone on CCA – diatoms from the second to fifth week (24.4 ± 1.9 μm/day; mean ± SE) was significantly lower than that of individuals on CCA + diatoms and the younger post-larvae on CCA – diatoms reached only 1 mm shell length in 5 weeks. The older post-larvae also did not grow well (27.8 ± 3.3 μm/day; mean ± SE) on CCA – diatoms and did not reach 3 mm by the end of the experiment. On CCA + diatoms, 74.6 ± 2.0 % (mean ± SE) of the younger post-larvae survived for the 5 weeks experiment. In contrast, the survival rate of the younger post-larvae reared on CCA – diatoms decreased rapidly until the second week, and 25.0 ± 12.6% (mean ± SE) of the individuals survived by the fifth week. The results of this study indicate that diatoms are essential for the rapid growth of post-larval abalone on the CCA. It is considered that the CCA *L. yessoense* itself is not a principal food source for post-larval *H. discus hannai* from approximately 500 μm to at least 3 mm shell length.

Introduction

The planktonic larvae of several species of the abalone genus *Haliotis* preferentially settle on crustose (non-geniculate) coralline algae (CCA) (Saito 1981; Morse and Morse 1984; Shepherd and Turner 1985; McShane and Smith 1988). At the time of metamorphosis, much of the yolk reserves is exhausted and post-larvae need to ingest an exogenous energy source immediately after metamorphosis (M. Asano and T. Kawamura 1992, unpublished). Thus CCA must provide food materials for the metamorphosed post-larvae. Some materials derived from CCA have been suggested to be possible food sources for post-larval abalone; surface mucus for *H. rufescens* Swaison 1822 (Morse and Morse 1984), cuticle and epithallial contents for *H. rubra* Leach 1814 (Garland *et al.* 1985) and outermost layer cells for *H. laevigata* Donovan 1808 and *H. scalaris* Leach 1814 (Shepherd and Daume 1996). Bacteria associated with CCA were suggested to be possible food

sources for *H. rubra* (Garland *et al.* 1985). Diatoms (Kawamura 1996) and trail mucus of juvenile and adult abalone (Takami *et al.* 1997) on CCA were also considered to be possible foods for *H. discus hannai* Ino 1953. Although it has been reported that juvenile *H. discus hannai* (Tomita and Tazawa 1971), *H. laevigata* and *H. scalaris* (Shepherd and Cannon 1988) from 5 to 10 mm in shell length feed directly on CCA themselves, the principal food sources for the post-larval abalone on CCA have not been elucidated yet.

In seed production hatcheries of abalone, benthic diatoms have been used as initial foods for post-larvae up to about 5 mm shell length. Recently the dietary value of benthic diatoms for the growth of post-larval abalone has been investigated in detail (Kawamura and Takami 1995; Kawamura *et al.* 1995). The results of these studies strongly suggest that benthic diatoms growing on CCA play important roles as food sources for post-larval abalone in the natural environment as well as in hatcheries (Kawamura *et al.* 1995; Kawamura 1996).

In the present study, survival and growth rates of post-larval abalone *H. discus hannai* reared on a CCA *Lithophyllum yessoense* Foslie 1909 with diatoms (CCA + diatoms) and without diatoms (CCA – diatoms) were compared in order to know the contribution of diatoms on CCA as food sources for post-larvae and the dietary value of CCA themselves.

Materials and Methods

Rocks encrusted with a CCA, *L. yessoense* were collected subtidally at Eno-shima Island, Miyagi Prefecture, Japan. The size of rocks was: 42.0–62.5 mm in length; 28.6–50.8 mm in width and 18.3–41.5 mm in breadth. The rocks were transported in a running seawater tank on a boat within 3 hours and kept in a running seawater tank at Tohoku National Fisheries Research Institute (TNFRI; Miyagi, Japan). The visible epibenthos, epiphytes and debris on the rocks except CCA were scraped off carefully, and rocks with CCA were rinsed well with filtered seawater (0.45 μm Millipore filter) to reduce diatoms and other micro organisms on CCA (rinsed CCA rocks) just before the experiments. All rocks used in the experiment were completely covered with CCA.

The experiment was carried out twice (Experiment 1: November 1995, Experiment 2: June 1995) using different sizes of abalone. In Experiment 1, growth and survival rates of post-larvae were compared for 5 weeks from just after metamorphosis between two experimental treatments; CCA + diatoms and CCA – diatoms. These treatments were set up as follows:

CCA + diatoms: Rinsed CCA rocks in sand-filtered seawater, which includes diatoms but not visible organisms, to encourage contamination with naturally occurring benthic diatoms.

CCA – diatoms: Rinsed CCA rocks used in filtered seawater (0.45 μm) with 6 mg/l concentration of GeO_2 (GeO_2 seawater).

GeO_2 seawater was prepared by the method of Chapman (1973). GeO_2 inhibits growth of diatoms, but does not affect the growth of any other algae (Chapman 1973).

Larval abalone used in Experiment 1 were hatched out in November 1995 at the Akita Prefectural Hatchery Center (Akita, Japan) according to the procedures described by Uki and Kikuchi (1984). Four days after fertilization at 20°C, the veliger larvae were transported to TNFRI within 5 hours. It was previously confirmed that the transport of veliger larvae by the method used in this study did not affect larval behaviour and development (Kawamura and Kikuchi 1992). The rinsed CCA rocks were placed on the bottom of 500 ml glass beakers with 300 ml of sand-filtered seawater (CCA + diatoms) or GeO_2 seawater (CCA – diatoms) and one rock per beaker. Fifty 5-day-old larvae, which were ready to metamorphose (Seki and Kan-no 1981), were placed in each beaker. Three replicates were used for each experimental treatment. Larvae were reared for 5 weeks at 20 °C, 6000 lux with a 12 hL: 12 hD photo-cycle. The shell length of individuals selected at random (5–7 inds. per beaker) was measured using an inverted optical microscope every week (week 1– 5). Survival rate of post-larvae was also assessed by counting the number of dead individuals during the experimental period. At the time of observation, all post-larvae were carefully removed from the CCA rocks using a water jet from a pipette and the rocks in beakers were replaced with new rinsed CCA rocks. Seawater in the

beakers was also changed. Another three rinsed CCA rocks just before the beginning of the rearing experiment and three old rocks each used in CCA + diatoms and CCA – diatoms were randomly selected and the diatoms on their surface were scraped off and fixed in a 5 % formalin-seawater solutions. Cell counts and identifications of diatoms were done under a light microscope. The surface areas of CCA rocks were calculated by approximating their shapes to spheres or polyhedron, and the benthic diatom cell density on the rocks was estimated. At the end of the experiment, we examined the gut contents of some live post-larvae reared on CCA + diatoms and CCA – diatoms.

In Experiment 2, growth rates of abalone from approximately 1.5 mm shell length were compared between CCA + diatoms and CCA – diatoms under the same environmental conditions as Experiment 1, except for the method of treatment of the rocks. The rocks used in Experiment 2 were previously soaked for 8 days in sand filtered (CCA + diatoms) or GeO_2 seawater (CCA – diatoms) before use. Larval abalone used in this experiment were hatched out in May 1995 at the Marine Development Company Ltd. (Iwate, Japan) using the same method as Experiment 1. Post-larvae were reared at the TNFRI under the CCA + diatoms condition until June 1995 when the experiment started. Seven individuals were reared in each experimental treatment. The shell length of all individuals was measured every week. Simultaneously, rocks and seawater were changed and diatom cells on the rocks were counted. Measuring and rearing methods were the same as in Experiment 1, but no replicates were conducted in Experiment 2. Gut contents of some live individual abalone on CCA + diatoms and CCA – diatoms were examined at the end of the experiment.

We evaluated the effects of GeO_2 on the rate of metamorphosis and peristomal shell formation and post-larval growth. These rates for individuals reared in GeO_2 seawater were compared with those for individuals reared in filtered seawater (0.45 μm). Metamorphosis and peristomal shell formation

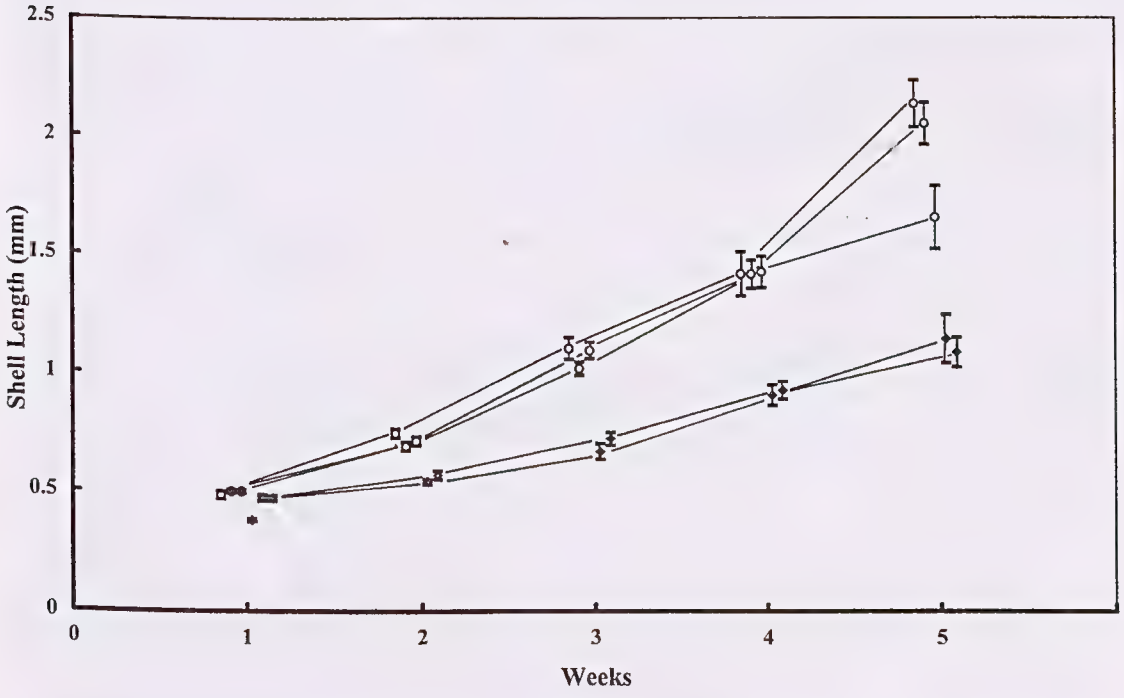

Figure 1. Growth of *Haliotis discus hannai* post-larvae (newly metamorphosed) fed on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data are mean shell length of post-larvae (± SE, n = 5-7) on each rock.

rates were measured as follows. Three replicates were tested for each experimental treatment (filtered seawater and GeO_2 seawater) using rocks encrusted with *L. yessoense* as substrata for larval settlement. Rocks were placed on the bottom of 500 ml glass beakers with 300 ml filtered seawater or GeO_2 seawater. Fifty 5-day-old larvae (siblings of those used in Experiment 1) were placed in each beaker. Forty-eight hours after the introduction of larvae to the beakers, the number of individuals that had metamorphosed and formed peristomal shells was counted using an inverted microscope. The effect of GeO_2 on post-larval growth was also evaluated in three different size of post-larval abalone approximately 1 and 2 mm in shell length (siblings of Experiment 1 animals) and newly metamorphosed post-larvae (siblings of Experiment 2 animals). These individuals were reared in filtered seawater or GeO_2 seawater on the conditioned plates with suitable food sources such as trail mucus of adult abalone and benthic diatom *Cocconeis scutellum* var. *parva* (Grunow) Cleve 1895 for post-larval abalone (Takami *et al.* 1997). After 7 or 15 days from the initiation of rearing, the shell length of individuals was measured.

All statistical analyses were carried out with the JMP (SAS Institute Inc. 1994) statistical computer package. Statistical tests of settlement and survival rates were carried out on arcsine-transformed data. This transformation helped to normalize the data and reduce heteroscedasticity. Untransformed values are presented in graphs and tables.

Results

In Experiment 1, most veliger larvae (5 days old) settled on both CCA + diatoms and CCA – diatoms rocks within 24 hours. Mean settlement rates of 3 replicates were 99.1 and 99.3 % on CCA + diatoms and CCA – diatoms, respectively. Subsequently, the post-larvae reared on CCA + diatoms

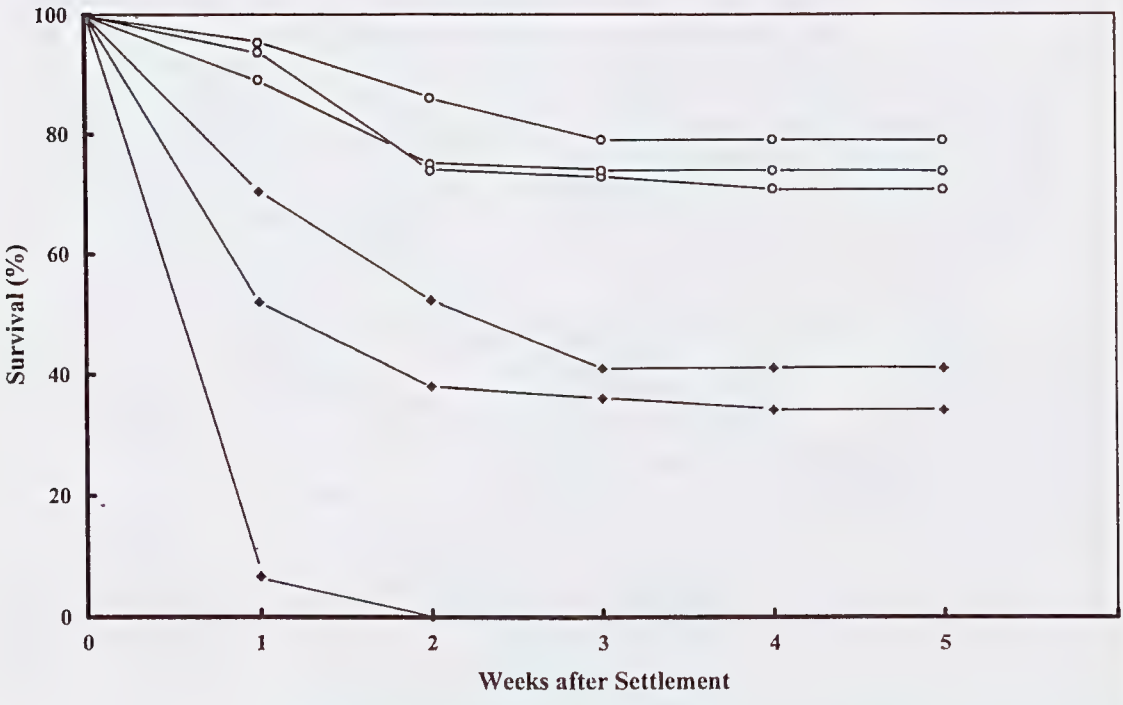

Figure 2. Survival of *Haliotis discus hannai* post-larvae reared on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data show the proportion of 50 added post-larvae surviving for each CCA rock.

Table 1. The mean densities of benthic diatoms colonizing on rocks (n=3) encrusted with CCA *Lithophyllum yessoeuse* used in the Experiment 1.

Diatom species	No. of cells (cells/mm², mean ± SE)		
	Before experiment	After experiement	
		Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2
Achnanthes spp.	ND	5.1 ± 2.6	ND
Amphora spp.	70.7 ± 26.3	432.0 ± 69.3	37.3 ± 22.5
Cylindrotheca closterium	ND*	230.1 ± 36.0*	ND*
Cocconeis spp.	11.1 ± 1.1	48.9 ± 9.1	9.0 ± 3.3
Navicula spp.	15.3 ± 4.1	48.8 ± 10.2	11.4 ± 9.7

ND: not detected

* There is a possibility that these values include cells of *Nitzschia longissima*. It was difficult to identify *Cylindrotheca closterium* and *Nitzschia longissima* by light microscopic observation.

grew well and reached about 2 mm shell length (53.4 ± 2.4μm/day; mean ± SE, Fig. 1) after 5 weeks. The post-larvae reared on CCA - diatoms reached 1 mm shell length at the end of Experiment 1. There was no difference in shell length of post-larvae reared on CCA + diatoms (496.3 ± 13.8; mean ± SE) and CCA – diatom (473.4 ± 20.2; mean ± SE) at week 1. However, the mean growth rate of individuals reared on CCA – diatoms from week 2 to week 5 (24.4 ± 1.9μm/day; mean ± SE) was significantly lower than that of abalone reared on CCA + diatoms (t-test, $p<0.01$; Fig 1). On CCA + diatoms, 74.6 ± 2.0 % (mean ± SE) of the individuals survived throughout the experimental period. In contrast, the survival rate of the individuals reared on CCA – diatoms was significantly lower by the second week (t-test, $p<0.01$). Only 25.0 ± 12.6 % (mean ± SE) of the individuals survived by the end of the experiment (Fig 2).

Figure 3 shows the growth of post-larval abalone (> ca. 1.5 mm in shell length) reared on CCA + diatoms and CCA – diatoms in Experiment 2. On CCA + diatoms, individuals grew well during the

Table 2. The mean densities of benthic diatoms colonizing on rocks (n=3) encrusted with CCA *Lithophyllum yessoeuse* used in the Experiement 2.

Diatom species	No. of cells (cells/mm², mean ± SE)			
	Before experiment [a]		After experiement	
	Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2	Sand filtered seawater	Filtered (0.45 μm) seawater dissolved with GeO_2
Amphora spp.	214.3 ± 68.1	64.2 ± 24.1	141.3 ± 33.8	57.8 ± 15.8
Cocconeis spp.	59.7 ± 49.0	1.8 ± 1.8	42.3 ± 8.7	ND
Cylindrotheca closterium[b]	78.4 ± 41.6[b]	ND[b]	21.6 ± 6.5[b]	ND[b]
Navicula spp.	84.8 ± 40.1	ND	61.0 ± 27.2	ND
Nitzschia spp.	ND	ND	14.2 ± 14.2	ND
Melosira sp.	ND	ND	4.7 ± 4.7	ND

ND: not detected

[a] Rocks were stocked in sand filtered seawater or filtered seawater dissolved with GeO_2 for 8 days before use of experiment in Experiment 2.

[b] There is a possibility that these values include cells of *Nitzschia longissima*. It was difficult to identify *Cylindrotheca closterium* and *Nitzschia longissima* by light microscopic observation.

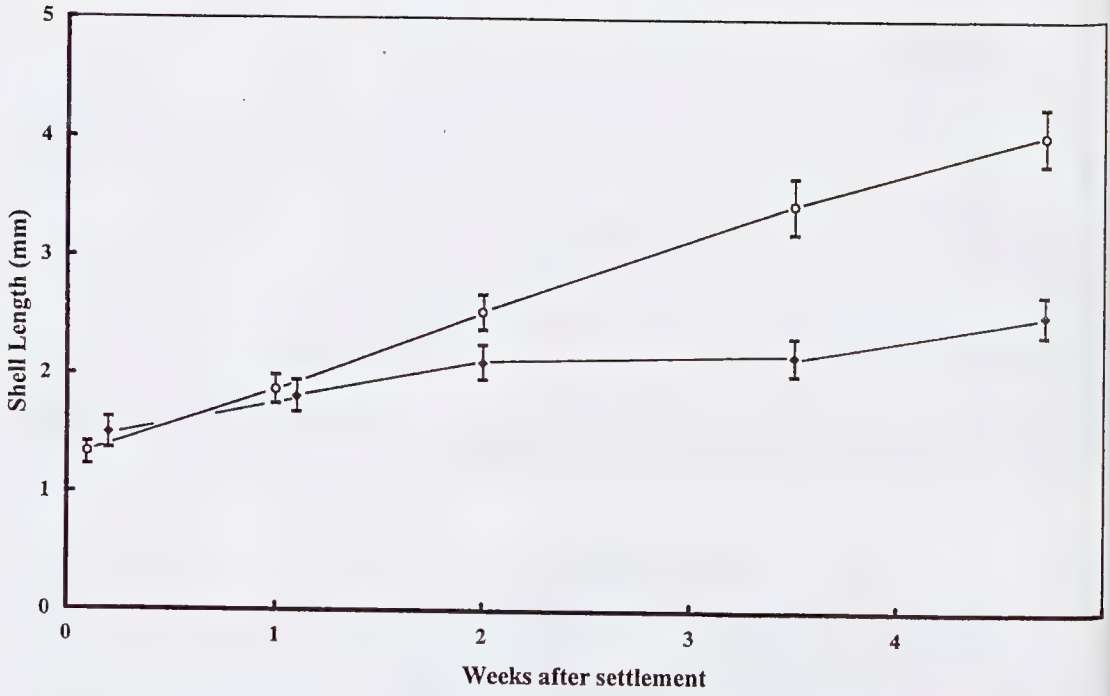

Figure 3. Growth of *Haliotis discus hannai* post-larvae (one month old) fed on CCA + diatoms (open circle) and CCA – diatoms (closed diamond). Data show mean shell length of post-larvae (± SE, n = 5-7) on each rock.

experimental period (85.0 ± 4.0 μm/day) and reached 4 mm in shell length at the end of the experiment after 33 days. In contrast, the mean growth rate of the individuals reared on CCA – diatoms (27.8 ± 3.3 μm/day) was significantly lower than that on CCA + diatoms throughout the experiment (t-test, $p<0.05$) and did not reach 3 mm. On CCA + diatoms, no dead individuals were observed during the experimental period whereas 2 out of 7 individuals died on CCA – diatoms by the end of the experiment.

The mean densities of diatom species on the rocks are shown in Table 1 and 2. Many diatoms were attached to CCA + diatoms rocks even after 1 week rearing of abalone. In contrast, diatom densities were very low throughout the experiment on CCA – diatoms rocks (Table 1, 2). Quantitative analyses of the feeding of post-larval abalone could not be carried out, but many diatom cells were found in the gut of post-larvae reared on CCA + diatoms whereas there were few cells in the gut of individuals on CCA – diatoms.

Table 3. Effect of rearing seawater with 6 mg/l concentration of GeO_2 on the metamorphosis and peristomal shell formation rates of larval abalone *Haliotis discus hannai*.

Rearing seawater	metamorphosis rate (%, mean ± SE)	peristomal shell formation rate (%, mean ± SE)
Filtered (0.45 μm) seawater	90.8 ± 1.0	63.4 ± 1.7
Seawater dissolved with GeO_2	94.6 ± 2.1	59.1 ± 5.7

Table 4. Effect of rearing seawater with 6 mg/l concentration of GeO_2 on the growth of post-larval abalone *Haliotis discus hannai*.

Rearing seawater	Initial shell length (μm, mean ± SE)	Daily growth rate (μm/day, mean ± SE)	No. of individuals measured	Rearing duration (days)
Filtered (0.45 μm) seawater	396.1 ± 13.9	37.0 ± 0.7	18	7
Seawater dissolved with GeO_2	394.3 ± 11.4	40.9 ± 0.7	18	7
Filtered (0.45 μm) seawater	847.7 ± 55.0	26.0 ± 5.9	5	15
Seawater dissolved with GeO_2	915.5 ± 63.1	31.7 ± 3.0	5	15
Filtered (0.45 μm) seawater	1899.7 ± 48.5	65.4 ± 7.8	6	7
Seawater dissolved with GeO_2	1972.0 ± 43.0	51.2 ± 9.3	6	7

The mean rates of metamorphosis and peristomal shell formation of larval abalone reared in GeO_2 seawater were not significantly different from those reared in filtered seawater (t-test, $p>0.05$; Table 3). There were no significant differences in the growth rates of all size groups of post-larvae between filtered seawater and GeO_2 seawater (t-test, $p>0.05$; Table 4). No dead individuals were observed in any size groups of abalone. These observations indicate that GeO_2 has no effect on larval metamorphosis, peristomal shell formation and post-larval growth.

Discussion

The results of this study indicate that diatoms are important food sources for the growth of post-larval *H. discus hannai* on CCA *L. yessoense*. The differences in the growth rate of post-larval abalone between CCA + diatoms and CCA – diatoms observed in the two experiments are considered to be due to the differences in amount of food sources which were derived from diatoms. We previously found that newly metamorphosed abalone which were fed on loosely adhered diatom species such as *Navicula ramosissima* (Agardh) Cleve 1895 and *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin 1964 were able to grow well up to a size of approximately 800 μm (Kawamura and Takami 1995). Extracellular mucus of diatoms was suggested as an primary food source for these early post-larvae (Kawamura and Takami 1995). In contrast, post-larvae over about 1 mm shell length require high levels of absorption of diatom cell contents in addition to any extracellular mucus for favorable growth (Kawamura *et al.* 1995). Since post-larvae cannot digest diatom cell contents in their alimentary canal without first rupturing the cell wall with the radula, only highly adhesive diatoms (such as *Cocconeis* spp.) and species with weakly silicified cell walls (such as *Cylindrotheca closterium*) which are easily broken open when they are grazed, are considered to be good food sources (Kawamura *et al.* 1995). In the present study, many diatoms including *Navicula* spp., *C. closterium* and *Cocconeis* spp. attached to the rocks used in CCA + diatoms treatment. Thus, CCA + diatoms rocks included the suitable food sources derived from diatoms for both younger and older post-larval abalone; extracellular mucus of diatoms for younger post-larvae and cell contents of *Cocconeis* spp. and *C. closterium* for older ones.

Although the survival rate of post-larvae on CCA – diatoms was significantly lower than that of individuals on CCA + diatoms at week 1(Fig. 2), post-larvae on CCA – diatoms grew as well as those on CCA + diatoms up to 1 week after settlement (Fig. 1). In addition, they reached 1 mm shell length 5 weeks after settlement (Fig. 1). It was reported that post-larval *H. rubra* can rasp the surface of CCA removing cytoplasmic content of the epithallium, suggesting that post-larvae depend for nutrition mostly on the polysaccharide layer grazed from CCA (Garland *et al.* 1985). We did not confirm whether post-larval *H. discus hannai* could remove the polysaccharide layer of the coralline alga or not. However, it is likely that post-larvae on CCA – diatoms showed comparable growth with those on CCA + diatoms rocks up to 1 week after settlement and grew to 1 mm at the end of

Experiment 1 using food sources associated with the CCA because there were few diatom cells attached to CCA – diatoms rocks (Table 1).

In the natural environment, diatom species with strongly adhesive solitary forms such as *Cocconeis* spp. were the dominant benthic diatoms in areas dominated by CCA, mainly due to their high tolerance to grazing pressures (Kawamura *et al.* 1992; Kawamura 1994). *Cocconeis* spp. are suitable food sources for post-larval *H. discus hannai* over approximately 1 mm shell length (Kawamura *et al.* 1995). Daume *et al.* (1997) also observed that post-larval *H. rubra* started to feed on *Cocconeis* spp. from 18 days after larval settlement even on a CCA, *Phymatolithon repandum* (Foslie) Wilks & Woelkerling. However, this diatom species is not considered a suitable food source for younger post-larvae (<800 μm) because of its relatively small quantities of extracellular substances (Kawamura and Takami 1995) and the difficulty of ingesting and absorbing diatom cell contents (Kawamura and Takami 1995; Daume *et al.* 1997). In this study, many loosely attaching diatom species which supply enough extracellular mucus for younger post-larvae were found growing on CCA + diatoms rocks as well as *Cocconeis* spp. The diatom communities growing on CCA in the natural environment may differ from those on CCA + diatoms rocks used in this study, because the occurrence of loosely attaching diatom species is limited in the natural environment (Kawamura *et al.* 1992). Thus, the supply of extracellular mucus of diatoms is probably lower in the natural environment than in the CCA + diatoms treatment. Other possible food sources on CCA in the natural environment include trail mucus of gastropods such as adult abalone (Takami *et al.* 1997), bacteria associated with CCA (Garland *et al.* 1985) or the mucus (Morse and Morse 1984), polysaccharide layer (Garland *et al.* 1985) of CCA themselves.

From the results of this study, it is considered that possible food materials derived from the CCA *L. yessoense* itself such as the polysaccharide layer are not sufficient food sources for the rapid growth of post-larval *H. discus hannai* from at least 500 μm shell length. In contrast, Shepherd and Daume (1996) reported that a CCA *Sporolithon durum* (Foslie) Townsend & Woelkerling 1995 had no biofilm on the surface, perhaps due to the sloughing of epithallial cells. They suggested that this CCA supplied enough food for *H. laevigata* and *H. scalaris* of 1 – 2 mm shell length. The dietary value of CCA themselves for post-larval abalone may differ between CCA species and their growth forms (Daume *et al.* 1997). Shepherd and Cannon (1988) reported that fragments of CCA were the predominant food item in the gut of *H. laevigata* and *H. scalaris* of 5 – 10 mm shell length and were eaten until at least 35 mm in their natural habitats. No individual abalone below 5 mm long were found to have macro-algae or calcareous fragments of CCA in their gut (Shepherd and Cannon 1988). *H. discus hannai* also ingests CCA and macro-algae from approximately 5 mm in shell length (Tomita and Tazawa 1971). A shift in the feeding habit from diatom feeding to macro-algal feeding may occur at about 5 mm shell length. Thus, it is possible that juvenile abalone can grow by feeding mainly on macro-algae including the fragments of CCA from this size. Further studies on the dietary value of CCA for the growth of juvenile abalone over 5 mm shell length are needed.

Acknowledgments

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Early juvenile growth of the abalone *Haliotis australis* in culture

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Abstract

Experimentally cultured juvenile *Haliotis australis* were fed different diets and their growth was measured. For juveniles between 3 and 6 mm in length the best growth was achieved on diatoms but macroalgal and artificial diets were also suitable for sustaining growth. The size at which the diet should be changed from microalgae to macroalgae was determined as 7 mm. Two batches of juveniles grown in a V-shaped tank rearing system averaged 15.9 ± 0.53 and 17.5 ± 0.69 mm in length after 13 and 12 months respectively. Methods to enhance the early juvenile growth are discussed.

Keywords: *Haliotis australis*; weaning; growth; abalone.

Introduction

In New Zealand, research into abalone culture began in the early 1980's with work on *Haliotis iris*, the black-footed paua, (Tong 1982) and the first commercial farming of this species began in 1987 (Henriques *et al.* 1989). Commercial operators would prefer to farm an abalone with a lighter coloured foot and *H. australis*, the yellow-foot paua, has been considered a suitable species. The feeding (Poore 1972a), growth (Poore 1972b), and reproduction (Poore 1973; Wilson & Schiel 1994) of adult *H. australis* have been investigated but little work has been done on juveniles because of their low abundance in the field (McShane *et al.* 1994). Research on *H. australis* at the NIWA Aquaculture Research Centre in Wellington has led to the development of techniques to bring the broodstock into ripe condition, to spawn them, and to rear the larvae (Moss submitted).

Newly settled abalone can ingest diatoms as early as 2 days after settlement (Norman-Boudreau *et al.* 1986), a food which can be part or all of their diet up to and into adulthood (Paul *et al.* 1976). In the wild adult *H. australis* feed preferentially on the rhodophytes *Pterocladia lucida* and *Hymenocladia lanceolata* (Poore 1972a) but nothing is known about the juvenile diet. It is not feasible to grow or harvest sufficient quantities of these seaweeds for culturing *H. australis* but the red seaweed *Gracilaria chilensis* is considered suitable as it can be readily cultured and is eaten by juvenile abalone (Tong & Moss 1992). Manufactured diets (Hahn 1989) may also be suitable. For a farmed abalone it is important to enhance the growth at all stages and therefore information is required on the dietary requirements of each size group. This study was carried out to investigate the growth of early juvenile *H. australis* in culture and to determine the diets most conducive to fast growth for different sized juveniles.

Materials and methods

Growth in V-shaped tanks

Adult *H. australis* collected from the Wellington south coast between March and May in 1991 and 1992 were held at the NIWA Aquaculture Research Centre, and brought into spawning condition

using elevated temperatures and *ad libitum* feeding of mixed seaweeds (Moss submitted). The broodstock were stimulated to spawn with hydrogen peroxide (Tong *et al.* 1992) in October 1991 (batch 1) and September 1992 (batch 2). The larvae were reared in 500 l flow-through tanks (Tong & Moss 1992) and at 10 days post-hatch, were settled onto diatom-coated (*Navicula* sp.) acrylic plates in 2m long V-shaped tanks (Tong & Moss 1992) at ambient water temperature (10°–18°C). When the juveniles reached about 5 mm, the rhodophyte *G. chilensis* was added to the tanks to supplement the diatoms.

Growth was monitored for 12 months following settlement, by measuring *in situ* the length of 100–250 juveniles, sampled at random and at irregular intervals. All measurements are presented as mean lengths ± 1 se. During the 12 months some of the juveniles (<1%) were removed for short-term growth and feeding experiments (see below) and not replaced. These removals reduced the density of juveniles in the tanks from about 1 juvenile per 28 cm^2 of tank surface to about 1 juvenile per 29 cm^2 but this should not have greatly biased calculations of growth in the tanks.

Growth on diatoms and seaweed

Growth of juvenile *H. australis* on two diets, microalgae (diatoms) and macroalgae (*G. chilensis*) was compared. Juvenile *H. australis* of 3 mm, 5 mm, 7 mm, and 9 mm length (with a range of ± 0.5 mm), were taken from 1 tank (batch 2) five months after settlement, tagged and assigned to 24 x 2 l flat-bottomed plastic containers. The tags were small pieces of coloured plastic attached to the shell with cyanoacrylate "super" glue (brand name "Loctite Prism 401"). The containers, which had a surface area of about 750 cm^2, were supplied with 18° ± 1°C seawater, filtered to the 1-5 micron range, at a flow rate of about 0.1 l min^{-1}. All treatments were maintained under a 12:12 photoperiod.

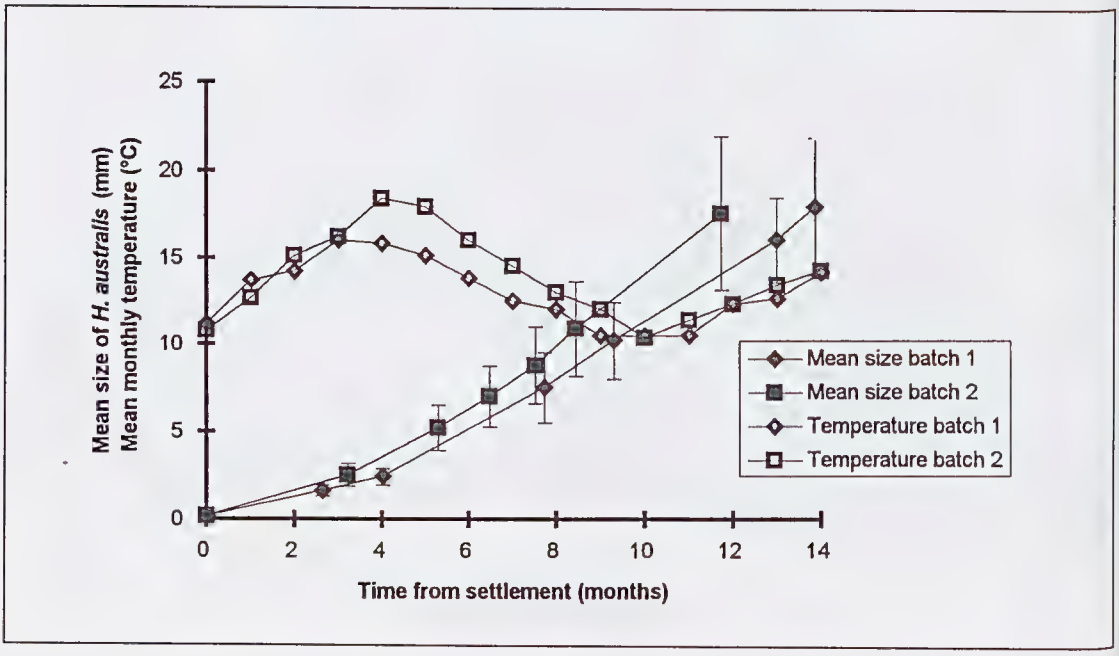

Figure 1. First year growth of juvenile *Haliotis australis* in semi-commercial V-shaped tanks at ambient temperatures at Mahanga Bay, Wellington. Batch 1 settled (September 1991) in one tank and batch 2 settled (October 1992) in two tanks. (All size measurements ± 1 S.D.).

Four individually tagged *H. australis* from 1 size group were assigned to each container with 3 replicate containers for each size group. The 24 containers (4 size groups x 2 diets x 3 replicates) were randomly positioned in an 8 x 3 grid.

In the microalgal diet, one 225 cm^2 acrylic plate, heavily coated with cultured diatoms (*Navicula* sp.) (Tong *et al.* 1992) was placed in each container. As the plates were grazed, further cultured diatoms were settled onto the plates in the containers to ensure a constant supply of food. Where the diatoms were grazed faster than they could be supplemented, the plates were substituted with others on which diatoms had been cultured. Care was taken to ensure that the *H. australis* transferred naturally from old plates to new. In the macroalgal treatment clean plates were placed in the containers along with whole fronds of the red seaweed *G. chilensis*. More *G. chilensis* was added during the experiment to ensure that the juvenile *H. australis* were fed *ad libitum*. The experiment was run for 30 days before the juveniles were remeasured and growth increments calculated.

Growth on diatoms, chopped seaweed and a manufactured diet

Growth of juvenile *H. australis* fed three diets was compared. The diets were: diatoms (*Navicula* sp.); *G. chilensis* which had been finely chopped in a domestic blender; and a powdered manufactured diet, brand name Makara (Walker 1993). Thirty-six 3 mm, 6 mm, and 9 mm (with a range of ± 0.5 mm) *H. australis*, were taken from the second tank (batch 2), tagged (as above). Four individually tagged juveniles of one size group were randomly assigned to each 2 l plastic container and there were three replicates of each treatment. The 27 containers (3 size groups x 3 diets x 3 replicates) were randomly positioned in a 9 x 3 grid and conditions were maintained as described above.

The microalgal (diatom) diet was fed as described above. The containers with *G. chilensis* were cleaned by siphoning every 3–4 days and the freshly chopped seaweed added and allowed to settle. Likewise, the manufactured diet was added every 3–4 days and allowed to settle on plates after the containers had been siphoned.

The experiment was terminated after 29 days and the juveniles remeasured and growth increments calculated and compared. ANOVA's were used to test treatment effects with Student-Newman-Keul (SNK) tests used to test differences between specific treatment means.

Results

Growth in V-shaped tanks

The juveniles settled in the V-shaped tanks in 1991 (batch 1) were first measured after 11 weeks when they had mean size of 1.6 ± 0.03 mm and again after 17 weeks when they were 2.6 ± 0.05 mm (Fig. 1). Those settled in 1992 (batch 2) reached a mean size of 2.6 ± 0.08 mm after 14 weeks. Growth rates over the first 3–4 months were between 20 and 30 microns per day for both batches. In both years high mortality was observed at about 4–5 weeks post-settlement, when there was a major decrease in the numbers of post-larvae in the tanks. This was confirmed when empty shells were collected from the bottom of the V-shaped tanks and measured. Their lengths ranged between 0.24 and 1.28 mm in length (mean 0.80 ± 0.05 mm). The first respiratory hole formed when the juveniles were 1.7 ± 0.01 mm in length (c. 8–10 weeks).

Growth rates between measurements for the first 6 months of monitoring ranged from 18 to 45 microns per day (batch 1) and from 24 to 49 microns per day (batch 2). Only after 6 months of age when the *H. australis* were had reached a mean length of about 7–8 mm did growth rates exceed 50 microns per day. Growth rates between measures for the remainder of the year ranged between 51–60 microns per day (batch 1) and 56–75 microns per day (batch 2). Batch 1 averaged 15.9 ± 0.53 in length (40 microns per day) after 13 months, but batch 2 grew faster over the first year and averaged 17.5 ± 0.69 mm (47 microns per day) after 12 months.

Table 1. Mean growth increments (mm ± 1 s.e.) and percent survival for 4 size groups of juvenile *H. australis* fed on either diatoms or *Gracilaria chilensis* over a 30 day trial period at 18°C. < or > indicates a significant difference between diets, = indicates no significant difference using the Student-Newman Keul (SNK) test

Size group:	Treatment: Diatoms	*Gracilaria chilensis*	SNK test
3 mm	1.20 ± 0.094 (75%)	1.54 ± 0.089 (92%)	D=G
5 mm	2.36 ± 0.101 (100%)	1.93 ± 0.134 (100%)	D=G
7 mm	2.68 ± 0.113 (100%)	3.27 ± 0.125 (100%)	D<G
9 mm	1.70 ± 0.107 (92%)	2.90 ± 0. 251 (92%)	D<G

Growth on diatoms and seaweed

The juvenile *H. australis* grew between 1.0 and 4.2 mm over the 30 days of the trial, with treatment means ranging from 1.20 ± 0.094 to 3.27 ± 0.125 mm (Table 1). Some mortalities occurred with both diets (Table 1) so the analyses of growth were done using data for only three *H. australis* per replicate. In those where all four had survived, data for one individual selected at random was removed to ensure the analyses remained balanced.

There was a significant difference in growth increment with size ($df = 3$; $F = 17.68$; $p = 0.001$) and there was a significant diet*size interaction ($df = 3$; $F = 12.99$; $p = 0.001$). The SNK tests showed there were no significant differences in growth of the 3 mm or 5 mm groups of *H. australis* fed the two diets (Table 1). However the 7 mm and 9 mm *H. australis* fed *G. chilensis* grew significantly faster than those fed diatoms.

Growth on diatoms, chopped seaweed and a manufactured diet

The mean growth of juvenile *H. australis* in all treatments was between 0.60 mm and 2.81 mm over the 29 days of the trial (Table 2).

Some mortalities did occur, particularly in the treatments which were being cleaned and fed every 3–4 days (Table 2). Analyses of growth increments used only three *H. australis* per replicate as explained above.

There were significant differences with diet ($df = 2$; $F = 15.15$; $p = 0.000$) and size ($df = 2$; $F = 16.27$; $p = 0.012$) and a significant diet*size interaction ($df = 4$; $F = 3.05$; $p = 0.044$). The SNK tests showed that the 3 mm and 6 mm *H. australis* fed on diatoms had significantly greater growth increments than the same sized juveniles fed either the finely chopped *G. chilensis* or the manufactured diet (Table 2). However there were no significant differences in the growth increments of the 9 mm *H. australis* fed any of the three diets.

Table 2. Mean growth increments (mm ± 1 s.e.) and percent survival for 3 size groups of juvenile *Haliotis australis* fed on either diatoms, finely chopped *Gracilaria chilensis*, or manufactured diet (brand name "Makara") over a 29 day trial period at 18°C. < or > indicates a significant difference between diets, = indicates no significant difference using the Student-Newman Keul (SNK) test

Size group:	Treatment: Diatoms	Chopped *G. chilensis*	Manufactured diet	SNK test
3 mm	1.83 ± 0.124 (100%)	0.78 ± 0.206 (83%)	0.60 ± 0.231 (67%)	D>G=M
6 mm	2.81 ± 0.111 (100%)	1.38 ± 0.238 (75%)	1.78 ± 0.213 (100%)	D>G=M
9 mm	2.40 ± 0.148 (100%)	2.20 ± 0.212 (100%)	1.93 ± 0.198 (100%)	D=G=M

Discussion

In culture systems, diatoms are the principle food source for post-larval and early juvenile abalone up to a size of about 5–10 mm (Ebert & Houk 1984; Hahn 1989; Hooker & Morse 1985; Seki 1980). This study showed there was no difference in growth and survival of 3 mm or 5 mm juvenile *H. australis* fed either diatoms or whole *G. chilensis*. However, growth on an artificial diet and on finely chopped *G. chilensis* was significantly slower than on the diatoms. The small (3–6 mm) *H. australis* fed whole *G. chilensis* grazed over the surface of the seaweed feeding on the epiphytes and diatoms associated with the surface of the seaweed and ate little of the seaweed itself. This type of feeding behaviour has previously been observed for juvenile *H. iris* fed epiphytised *G. chilensis* (Pickering 1990).

Larger juveniles are fed seaweeds because they require more diatoms than can easily be produced (Uki *et al.* 1981; Ebert & Houk 1984). The 7 and 9 mm juvenile *H. australis* fed *G. chilensis* ate the seaweed as well as the epiphytes growing on it. The 9 mm juveniles in the second trial showed no significant differences in growth between the three diets fed, however the 9 mm juveniles in the first trial fed diatoms grew significantly slower than those fed *G. chilensis*. A possible explanation for the slower growth is that there were insufficient diatoms for the number and size of juveniles in the first trial. Additional diatoms that were added to plates and allowed to settle were rapidly consumed. In the second trial sufficient diatoms were available so they were not limiting.

The calculated growth rates of *H. australis* in the V-shaped tanks were generally lower (18–75 microns per day) than in the experimental trials fed either diatoms (40–100 microns per day) or *G. chilensis* (50–110 microns per day). Higher overall water temperature and better availability of suitable food probably contributed to the faster growth of the juveniles in the small scale trials. Temperature may also have accounted for the differences in growth seen between the two batches of juveniles in the V-shaped tanks as the ambient temperatures in 1992 (batch 2) were higher than those in 1991 (batch 1).

In the V-shaped tanks the small pennate diatoms, most suitable as food for post-larval abalone (Norman-Boudreau *et al.* 1986; Hahn 1989), were rapidly grazed and replaced by overstory diatoms, which are less suitable as food (Matthews & Cook 1995). The addition of *G. chilensis* when the juveniles reached about 5 mm did not immediately increase their growth rate. Turbulence created by aeration in the V-shaped tank caused the *G. chilensis* to circulate and the 5 mm juveniles were unable to climb onto the seaweed to feed on the epiphytes as they did in the experimental containers. The macroalga may also have inhibited diatom growth in the V-shaped tanks by stripping the nutrients from the water (Pickering 1990). Once the juveniles reached 7–8 mm in the V-shaped tanks they were able to catch and ingest the *G. chilensis* and growth rates increased to more than 50 microns per day. This is still lower than the growth rates of similar sized juveniles from the experiments (60 –110 microns per day) indicating that the feeding systems in the V-shaped tanks can be improved. In a commercial system best growth will be achieved by weaning the 7–9 mm juveniles on to either macroalgal or manufactured diets because of the difficulties associated with maintaining a diatom coat.

The most appropriate diet for *H. australis* culture will depend on the availability and cost of various foods, the size and number of juveniles to be grown and the tank design to be used. Tanks that are suitable for feeding and growing diatoms may not be suitable for feeding out seaweeds or artificial foods. The V-shaped tanks we use have a high surface area (11.5 m^2) for growing diatoms and for the juveniles to graze over (Tong & Moss 1992). They are also suitable for feeding out seaweeds once the juveniles are large enough to catch the seaweed (Tong *et al.* 1992). Flat-bottomed settlement tanks of a similar volume to the V-shaped tanks have a much smaller surface area (3.8 m^2) and are less productive at growing diatoms and juvenile abalone (Ebert & Houk 1984; Tong & Moss 1992) but may be more suitable for feeding manufactured powdered foods (Ewing pers comm.) which settle out on the horizontal surface.

Careful management of diatom supply, and changing the diet of juveniles from diatoms to seaweeds or manufactured diets at the appropriate size will enhance the early growth of *H. australis* in culture.

Other factors such as water temperature (Leighton 1974; Leighton *et al.* 1981; Uki *et al.* 1981; Hahn 1989) and species (Uki & Kikuchi 1979) and nutritive value of the diatoms (Austin *et al* 1990) have also been shown to affect the growth rates of abalone. These factors, and the interactions between them, will also affect the early growth of juvenile *H. australis* and need to be investigated.

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Differences in the relative abundance of abalone (*Haliotis iris*) in relation to the perceived status of two regional fisheries in New Zealand

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Abstract

Population survey results and commercial catch data were obtained for two fisheries (PAU 5 & PAU 7) for the New Zealand abalone *Haliotis iris*. PAU 5 had greater catch rates and greater relative abundance of harvestable abalone (≥ 125 mm shell length) than areas of PAU 7, but PAU 5 is perceived by participants to be vulnerable whereas PAU 7 is perceived as sustainable. Contrasts in the relative abundance of juveniles (abalone in cryptic habitat < 100 mm shell length) and pre-recruits (≥ 100, < 125 mm) appear to correlate with the perception of participants on the sustainability of the two fisheries. For areas within individual fisheries, population surveys in 1993 and again in 1995/96 revealed no differences in the relative abundance of harvestable individuals. However, increases in the frequency of juveniles, pre-recruits, and large patches (≥ 5 individuals) were recorded for two of the three areas surveyed in PAU 7. No differences in any of these indices were found for PAU 5. These results suggest that perceptions of the sustainability of the New Zealand abalone fisheries relate to the relative levels of pre-recruits, particularly those individuals in large aggregations, rather than the abundance of harvestable individuals.

Keywords: *Haliotis iris*, sustainability, diver behaviour, abundance, aggregations.

Introduction

Reviews of methodology for the assessment of abalone (*Haliotis* spp.) fisheries have emphasised the practical difficulties of estimating stock size and appropriate yields (Sloan and Breen 1988; Breen 1992; McShane 1997). These difficulties relate to the disaggregate stock structure of abalone (Shepherd and Brown 1993), and problems of censusing populations in shallow wave-exposed coastal habitats (McShane 1995).

All Australasian abalone fisheries are managed under a combination of output (catch quotas), and input (size limits) controls (Shepherd 1992). Although considerable research has been undertaken, management actions, and evaluations of management strategies, have been based more on qualitative assessments related to the perceptions of participants in the fishery, rather than on formal quantitative assessments (Shepherd 1992). The considerable commercial value of abalone fisheries demands defensible assessments and fishery-independent assessments are presently being undertaken in most Australasian abalone fisheries.

In New Zealand, quantitative assessments linked to population surveys (*Haliotis iris* Martyn) have been conducted in two major regional fisheries: PAU 5, encompassing southern New Zealand; and PAU 7, the top of the South Island including D'Urville Island (McShane *et al.* 1994). Together,

these fisheries account for more than half of the total annual catch of abalone from New Zealand (about 1200 t). Although not quantified, the general perception of the status of these regional fisheries differs considerably between participants in the two fisheries. PAU 5, the largest fishery with a total allowable commercial catch (TACC) of 442.8 t, is considered by many participants to be vulnerable and, reflecting this concern, participants successfully applied for a 10% reduction in the TACC in 1994. In contrast, PAU 7 is generally considered by participants as capable of sustaining the current TACC of 266.2 t.

Here, I present information on commercial catch data and population survey results for the two fisheries in the context of the contrasting perceptions of the participants. I discuss the behaviour of abalone divers as an important factor in determining the nature of the impact of fishing on abalone populations, and I examine the utility of alternative abundance indices in assessing stocks of abalone.

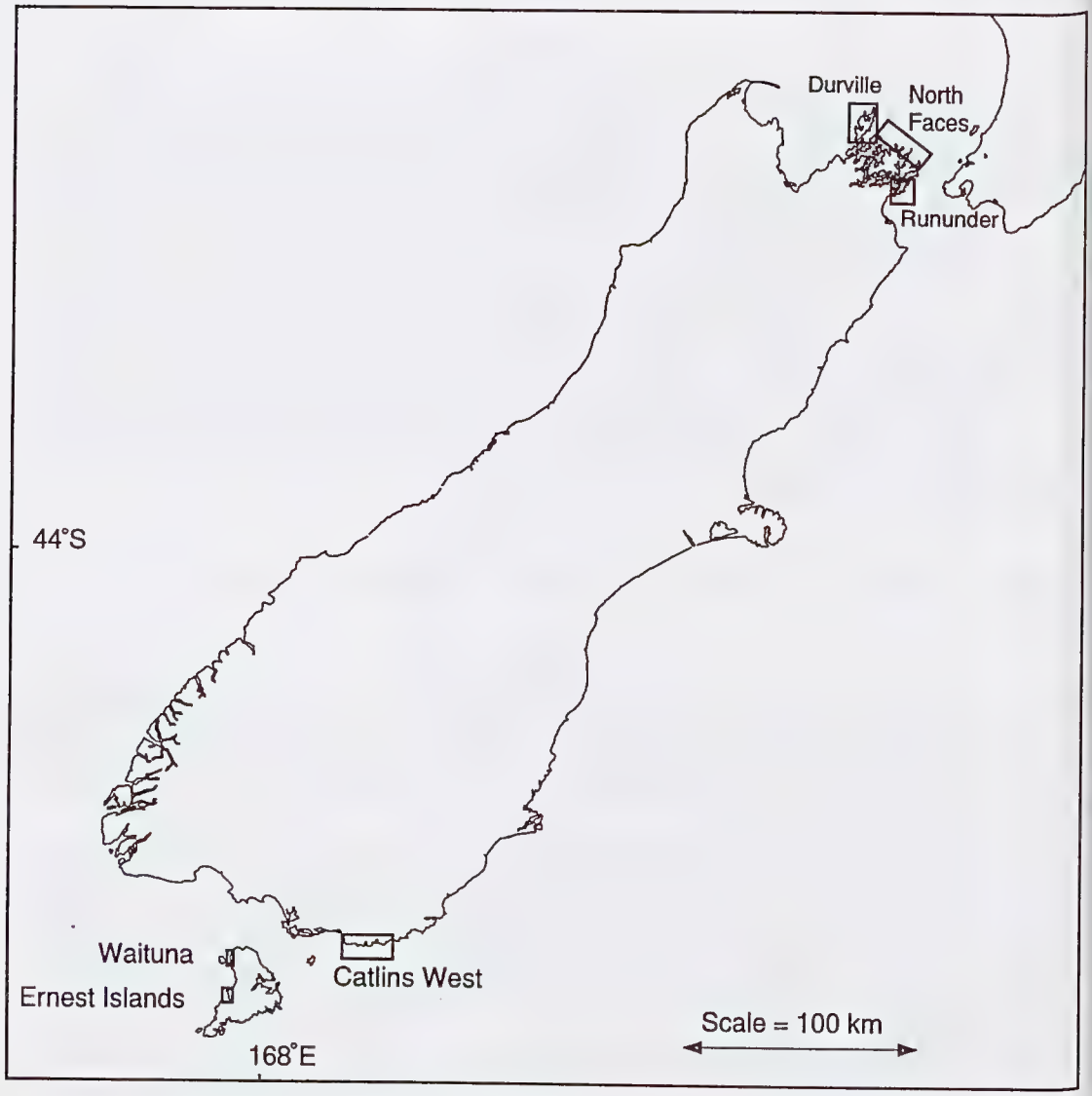

Figure 1. Locality map for areas surveyed in PAU 5 and PAU 7.

Materials and Methods

Commercial catch data were obtained from compulsory log books submitted by fishers. Catch and effort data are collected over lengths of coastline (about 100 km) which are either larger than, or overlap, those coastal areas considered for population surveys. This spatial subdivision of reporting requirements for catch and effort data reflects a uniform subdivision for reporting of all coastal fisheries in New Zealand and the utility of catch and effort data, other than reporting gross annual trends, is considered poor for the New Zealand abalone fishery (McShane *et al.* 1996).

Three areas were chosen for survey in PAU 5 and PAU 7 based on coastal habitat nominated by abalone divers as being important to their respective fisheries. Of these, Catlins (PAU 5), and D'Urville, North Faces, and Runander (PAU 7) had successive population surveys undertaken. Two other areas in PAU 5 (Waituna, Ernest Islands) were considered as being subject to high exploitation rates (McShane *et al.* 1996) and were included for comparison with the other areas surveyed. Areas were about 20 km of coastline, except for D'Urville, North Faces, and Runander which represent approximately 34, 88, and 35 km of coastline respectively. Access and weather largely determine the frequency of fishing in each area. The three areas surveyed in PAU 5 are often exposed to heavy seas, whereas the areas surveyed in PAU 7 may be considered as relatively sheltered (McShane *et al.* 1994).

For the purposes of survey, the coastline of each area was subdivided into 250 m strips and each strip was considered a potential sample site. Sites were allocated randomly and the number of sites sampled within each area was based primarily on its estimated available habitat, and on the variance in abundance of abalone estimated from preliminary surveys conducted in 1991 and 1992 (McShane *et al.* 1994). For areas of PAU 7, and Catlins in PAU 5, surveys were conducted 2–3 years apart according to sampling opportunities, and the perceived potential for measuring a significant fishing-induced change in the abundance of abalone in each area (McShane *et al.* 1996).

At each site two divers, one on each side of the research vessel, each searched for *Haliotis iris* on subtidal reef habitat for 10 min (McShane 1995). As *H. iris* has a preference for shallow habitat, divers searched habitat above 10 m depth. They used surface-supplied air which limited the searching area to a 100 m radius described by the air hose. Thus, at any one site, individual divers would sample two replicate subsites separated by tens of metres but by no more than 200 m. Two timed searches were made at each site and the frequency of patches of various sizes was recorded according to the method described by McShane (1995). Samples of no more than 4 individuals were collected from each patch encountered at each subsite.

Relative abundance data and the density of emergent abalone (i.e., those on open reef habitat visible to divers) were derived from patch frequency records. Patch size was classified according to the numbers of individuals per patch: patch1, 1–4; patch2, 5–10; patch3, 11–20; patch4, 21–40; patch5, 41–80; patch6, 81–160. Relative abundance data, derived from patch frequency data, were standardised to take into account searching and handling time as described by McShane (1996). All patches with 5 or more individuals were considered as large and targetted by abalone divers (McShane 1995). Estimates of the density of harvestable and pre-recruit abalone were made for each

Table 1. Summary data for surveys conducted for *Haliotis iris* in PAU 5 and PAU 7. Sample size (n) is the number of subsites surveyed on each occasion.

Area	Fishery	Date of first survey	$n_{(1)}$	Date of second survey	$n_{(2)}$
Runander	PAU 7	October 1993	32	June 1996	32
North Faces	PAU 7	April 1993	28	May 1995	30
D'Urville	PAU 7	April 1993	24	November 1995	24
Waituna	PAU 5	August 1993	19	February 1995	34
Ernest Islands	PAU 5	no survey	–	June 1995	39
Catlins	PAU 5	November 1993	20	November 1996	18

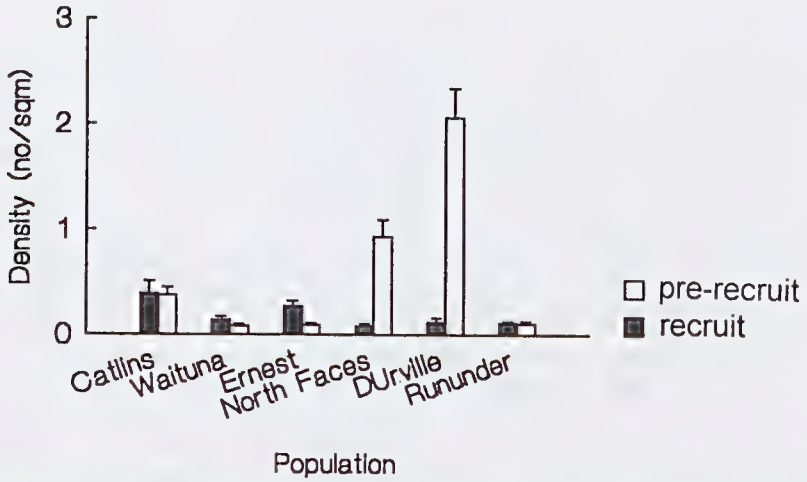

Figure 2. Comparison of the density of pre-recruit (under 125 mm) and harvestable *Haliotis iris* (= 125 mm) sampled from areas of PAU 5 and PAU 7. Data are means with s.e.

subsite by multiplying the density of emergent abalone by the relative proportion of individuals ≥ 125 mm, and individuals < 125 mm respectively.

A further 10 min was spent by each diver at each site searching for juveniles in under-boulder habitat. All specimens collected were measured for shell length to the nearest mm aboard the research vessel. Juveniles were not included in estimates of the relative abundance of harvestable or pre-recruit abalone. As the availability of juvenile habitat can vary from site to site, juvenile abundance data were considered semi-quantitative and are used mainly in expressing relative differences among areas within fisheries, and between fisheries, i.e., PAU 5 vs PAU 7.

Where appropriate, means of relative abundance data were compared by ANOVA after testing for homogeneity of variances with Cochran's test.

Results

Reliable catch and effort data were only available from 1989. There was interannual variation in catch rates in PAU 5 and PAU 7, but catch rates were consistently higher in PAU 5 than in PAU 7 (Table 2). There was no consistent trend in catch rates for either fishery.

There was significant variation in the relative abundance of *H. iris* among the areas surveyed during 1995/96 (Fig. 2). The areas surveyed in PAU 7 all had significantly lower densities of

Table 2. Total allowable commercial catch (TACC), catch, and catch per unit effort (CPUE) data for two fisheries for *H. iris* (PAU 5 and PAU 7). Data are shown for each fishery as catch (tonnes) and CPUE (kg per diver h).

	PAU 5			PAU 7		
Year	TACC	Catch	CPUE	TACC	Catch	CPUE
1989–90	459.5	459.5	67.7	263.5	248.6	40.7
1990–91	484.9	528.2	82.2	266.2	274.9	37.2
1991–92	492.1	486.8	58.2	266.2	275.1	35.1
1992–93	442.8	440.2	61.0	266.2	268.0	45.7
1993–94	442.8	440.4	56.6	266.2	255.5	46.2
1994–95	442.8	436.1	43.1	266.2	244.4	40.2

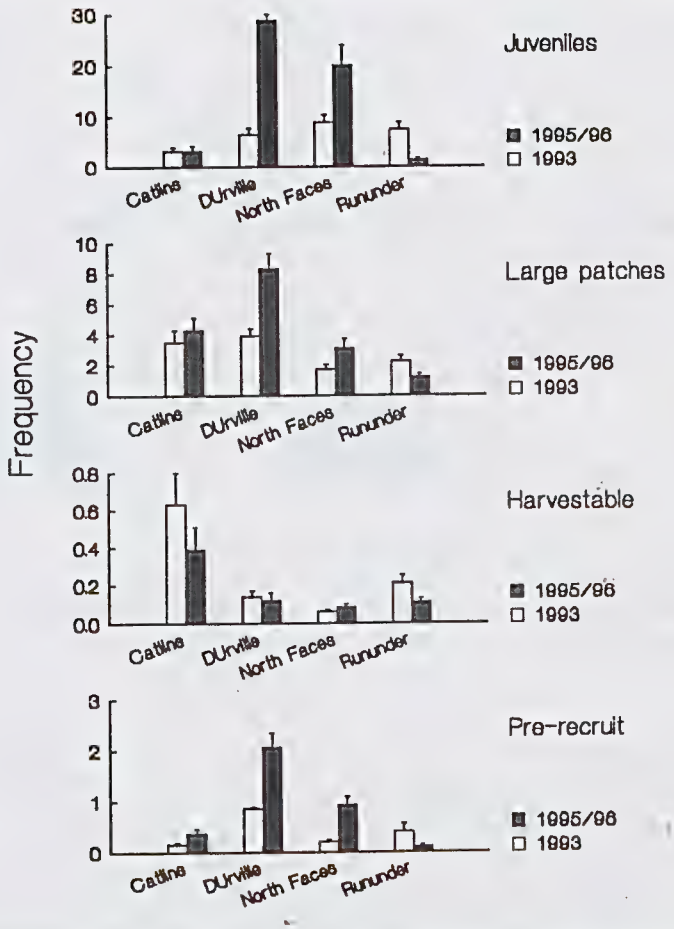

Figure 3. A comparison of the frequency of juveniles, large patches (≥ 5 individuals), harvestable individuals, and pre-recruit *Haliotis iris* in 1993 and 1995/96. Data are means with s.e. and are nos/10min (juveniles, large patches) or estimated nos/sqm (harvestable, pre-recruit).

harvestable individuals than Catlins or Ernest Islands in PAU 5 (by ANOVA $P < 0.05$). However, North Faces and D'Urville had significantly greater numbers of pre-recruits than the other areas surveyed.

There were significant increases in the frequency of juveniles recorded at D'Urville and North Faces, and a decrease recorded for Rununder in successive surveys (Fig. 3). There were increases in the frequency of large patches recorded for D'Urville and North Faces, but there was no significant change in the abundance of harvestable individuals for any of the areas surveyed (Fig. 3). However, increases in the abundance of pre-recruit abalone were shown for D'Urville and North Faces (by ANOVA, $P < 0.05$).

There were no obvious differences in the availability of juvenile habitat among localities within areas. Most sites had shallow boulder-strewn habitat which offered refugia for juveniles of *H. iris*. However, sites in areas of PAU 5 were clearly exposed to heavy wave action as most of the reefs surveyed showed evidence of mobility of boulders on shallow reef habitat. In contrast, most of the under-boulder habitat encountered on the shallow reefs in PAU 7 was colonised by crustose coralline algae and other biota typical of juvenile abalone habitat (c.f. Shepherd and Turner 1985).

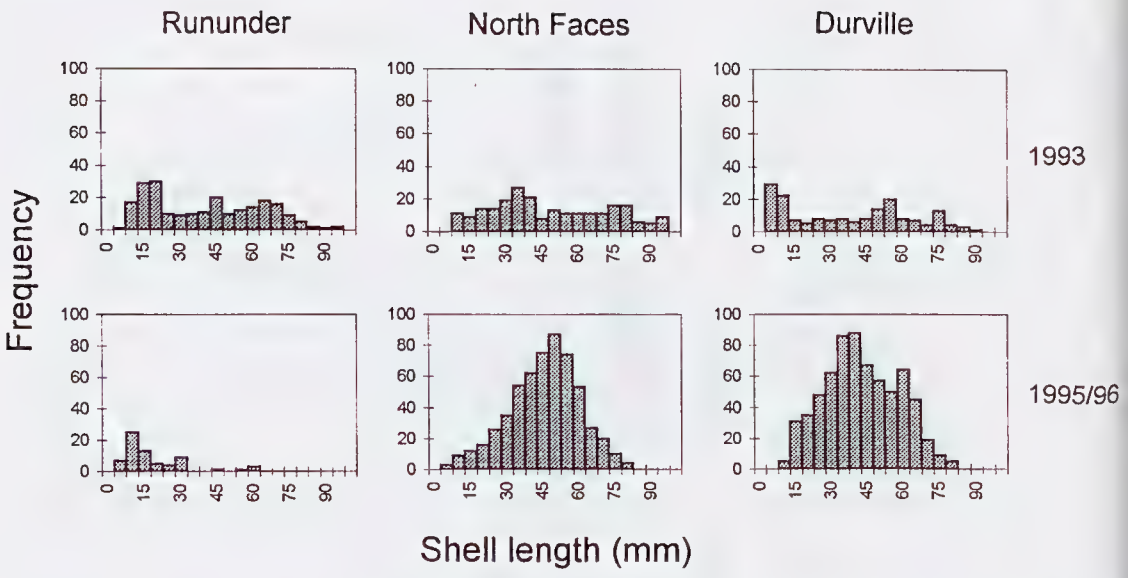

Figure 4. Length-frequency distributions of juvenile *Haliotis iris* sampled from under-boulder habitat in areas of PAU 7.

So few juveniles were found in PAU 5 that no length frequency distributions are presented. However, in PAU 7, cohorts of putative year classes were evident from length frequency distributions from the three areas (Fig. 4). A strong cohort of individuals 10–20 mm in length was apparent from Runander and, for 1993, D'Urville. The decrease in the frequency of juveniles at Runander in 1996 was associated with the loss of putative 2+ and 3+ year classes from the population (see McShane and Naylor 1995). Similarly, the strong increase in the frequency of juveniles and pre-recruits recorded for D'Urville and North Faces was associated with evident progression of juvenile year classes from 1993, and settlement in the years between the surveys (Fig. 4).

Discussion

Catch rates and estimates of relative abundance of harvestable abalone are inconsistent with the perceptions by participants of the status of two major regional abalone fisheries in New Zealand. Clearly perceptions of fishers are influenced by a range of factors including experience, motivation, and future expectations. Exploration of these factors is beyond the scope of this study, but differences in them may be expected among participants in the two fisheries. Perceptions of participants are discussed only in the context of their contrasting assessment of the status of two regional abalone fisheries and are described in relation to differences in the relative abundance of abalone.

Changes in the relative abundance of *Haliotis iris* were recorded in successive surveys over a two to three year period. However, these changes were associated with differences in the relative abundance of juveniles and pre-recruits rather than differences in the frequency of harvestable individuals.

There are two possible reasons for differences in the perceptions of participants in PAU 5 and PAU 7. First, fishing effort in PAU 5 has been traditionally concentrated in a relatively small spatial area of the fishery; Stewart Island (McShane *et al.* 1994). The perceptions of participants may reflect their experience of Stewart Island in a heavily fished area such as Waituna rather than a lightly fished area such as Catlins (McShane *et al.* 1996). Second, there are obvious differences in the

recruitment potential among the two fisheries. Despite intensive searches of juvenile habitat, most sites surveyed in PAU 5 had no juveniles at all (McShane *et al.* 1996), and there were relatively few pre-recruits among the emergent abalone sampled. For North Faces and D'Urville there was a tenfold or greater difference in the relative abundance of juveniles than in areas surveyed in PAU 5 (McShane *et al.* 1994), and the size composition results suggested strong year classes present in areas of PAU 7. Furthermore, there was a significant increase in the abundance of pre-recruits for North Faces and D'Urville from 1993 to 1995 which was associated with an increase in the frequency of large patches.

A general pattern emerges from the survey results which suggest real differences in the productivity of the two fisheries. PAU 5 has mainly unproductive stocks characterised by an accumulation of large individuals and an infrequency of pre-recruits and juveniles (McShane *et al.* 1994). In contrast, consistent recruitment to the harvestable stock in PAU 7 may be expected from strong juvenile year classes and abundant pre-recruits. Thus, populations in PAU 7 are more likely to sustain regular fishing, but with a lower catch rate, than those in PAU 5.

Diver behaviour is clearly important in determining the impact of fishing on abalone populations. Simulation models suggest that selective fishing of aggregations (large patches) is a conservative fishing strategy because the harvest of disaggregated individuals involves substantial searching time (McShane 1996).

Realistic and spatially explicit models, which incorporate the aggregational structure of abalone populations and include the selective fishing behaviour of abalone divers need to be developed for abalone fisheries (see Allen and MacGlade 1986 for fish). Indices of juvenile and pre-recruit abundance, which may be important in shaping perceptions of abalone divers and hence their behaviour, will lead to better evaluations and more objective decision making in the management of abalone fisheries than the historical reliance on qualitative information.

Acknowledgments

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Optimum conditions using manganese as a shell marker for abalone age validation studies

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Keywords: manganese; time-marker; abalone; shell; cathodoluminescence; age validation

Abstract

Blacklip abalone, *Haliotis rubra* (L.), were immersed for 48 to 144 hours in buckets containing sea water to which $MnCl_2.4H_2O$ was added at concentrations ranging from 9.5 to 1890 mg L^{-1}, in order to determine the optimum concentrations for marking. Manganese concentrations above 945 mg L^{-1} over the immersion period were lethal to abalone. Cathodoluminescence microscopy was used to detect manganese mineralised within shell layers *in situ*. Although the average length and width of manganese carbonate marks at the outer growing margin of the abalone shell increased with higher concentrations, marks in the shell area under the spire appeared to be maximal at intermediate concentrations. Better marks were found at the growing edge than under the spire, although this appeared to depend on the seasonal timing of marking. Manganese concentrations of 378 mg L^{-1} or less in sea water for 48 h were not lethal to abalone after 10 days, but the scores of marks indicated a possible sub-lethal toxic effect of 144 h immersion at 272 mg L^{-1}. In a field trial we obtained 93% survival after 14 days of abalone exposed to 200 mg L^{-1} of manganese for 48 h and of these 60% showed marks in the spire region, where age is estimated, so this technique can be used in age validation studies. However, the effectiveness of manganese as a time-marker for increment analysis appears to depend on the growth of abalone when exposed to the marker and the ambient levels of manganese in the natural habitat.

Introduction

Layers formed in the calcified tissue of aquatic animals, such as otoliths of fishes and shells of gastropods, are often used to age individuals but, in each case, the temporal nature of growth increments requires validation (Beamish and McFarlane 1983). This is important for all species that are commercially harvested, as accurate age estimation allows for assessment of populations using age specific models, such as egg-per-recruit and yield-per-recruit (Day and Fleming 1992: Nash *et al.* 1994). Darker layers under the spire of abalone were suggested to be annual by Muñoz-Lopez (1976), and have been used to estimate the age in a number of species (Prince *et al.* 1988; Nash *et al.* 1994; Shepherd *et al.* 1995a; b), but without proper validation (Day and Fleming 1992). In Australia, the management of abalone stocks will remain somewhat subjective until the age structure of populations can be determined with confidence, as this will allow better estimates of population parameters such as mortality, and facilitate the use of age structured models.

Cross-sections cut through abalone shell expose a calcified outer prismatic and inner nacre layer (Nakahara *et al.* 1982; Mutvei *et al.* 1985; Dauphin *et al.* 1989). The nacre layer consists of stacks of brick-like tablets of aragonite with thin protein-sheets between the laminae. These stacks of laminae are interspersed with thick dark protein rich layers (Erasmus *et al.* 1994; Shepherd *et al.*

1995b). In the spire region, under the apex, the protein layers form "rings" (similar to rings in a tree) when the spire is ground down, and appear to be deposited regularly, between one and four times per year in different species (Muñoz-Lopez 1976; Prince *et al.* 1988; Nash *et al.* 1994; Shepherd *et al.* 1995a; b). Similar protein layers are laid obliquely near the growing margin (the edge of the body whorl) and correspond to growth checks in many species (Day and Fleming 1992). These growth checks consist of a thick layer of conchiolin protein material and either aragonite or calcite (Zaremba *et al.* 1996; Hawkes *et al.* 1996). They have been shown to be formed annually in *H. discus discus* from Japan (Sakai 1960; Kojima 1995), in *H. mariae* from Oman (Shepherd *et al.* 1995a) and perhaps also in other abalone species (Forster 1967; Poore 1972). However, they appear to be deposited irregularly in other species, and may be induced by seasonal, reproductive and/or disturbance events (Sakai 1960; Day and Fleming 1992). The timing and frequency of deposition of these growth checks within the nacreous layer of both the spire and edge regions require validation (Day *et al.* 1995).

To date, validation of the temporal nature of growth increments under the spire is based on the rings found in size classes of juvenile abalone assumed to be annual cohorts, or simply by comparing the graph of size versus number of rings to predicted growth curves (Prince *et al.* 1988; Shepherd *et al.* 1995a; b). This method cannot validate age classes beyond maturity because as growth slows, size no longer provides any information about age. Without proper validation of the periodicity of growth layers, the age structure of populations cannot be estimated with any confidence (Day and Fleming 1992; McShane and Smith 1992; Erasmus *et al.* 1994; Day *et al.* 1995).

Staining of calcified tissue with chemical tags, such as fluorochromes and strontium, has been used to validate the timing of growth layer deposition in order to determine ages of molluscs, sharks and fishes (Yamada 1971; Behrens and Mulligan 1982; McFarlane and Beamish 1987; Villiers and Sire 1985; Officer 1995). Pirker and Schiel (1993) and Day *et al.* (1995) have tested various fluorochromes to mark abalone shells, but although these stained the growing edge of abalone shells, they failed to stain the nacre under the spire where age is estimated (Day *et al.* 1995).

Behrens and Mulligan (1990) outlined the criteria for a chemical to be an effective tag. The stain must be non-lethal, rare in the animal and ambient medium, incorporated as a permanent mark in the body and detectable a year or more after marking. Behrens and Mulligan (1990) first suggested manganese may be useful as a chemical tag. They showed that manganese, introduced through artificial feed, could be detected in powdered bone samples of hatchery salmon, using X-ray fluorescence and microprobe analysis. However, the ability to detect a chemical tag *in situ* would increase its use as an effective chemical marker in increment studies. When a stain can be viewed within the calcified tissue it acts as a time-mark, so that the frequency of growth layers deposited after the mark can be investigated. Several paleontological studies have shown that natural trace levels of manganese in calcified tissue can be detected *in situ*, using cathodoluminescence (CL) (Sommer 1972; Barbin *et al.* 1991 a; Mazzoleni *et al.* 1995). Barbin *et al.* (1991 b) and Barbin (1992) used CL microscopy to detect manganese bound within growth layers of *Pecten* sp. and *Nautilus* sp. We have recently shown that immersion of abalone, *H. rubra*, in sea water enriched with manganese can be used to mark the growing edge of the shell. Manganese appears to substitute for calcium in the $CaCO_3$ lattice. The marks can be detected using CL, and reveal the mineralog of the layers by colour (Hawkes *et al.* 1996). Under CL, layers emit either an orange-red or yellow-green colour depending on whether manganese is incorporated into calcite or aragonite, respectively, because electrons are excited to different energy states in the two minerals (Sommer 1972). In this paper, we present the results of experiments to determine the optimum concentrations of manganese and immersion times required for this chemical marker to be useful for incremental analysis work on blacklip abalone for age validation studies.

Materials and Methods

Blacklip abalone, *Haliotis rubra*, were collected from Rickets Point, Port Phillip Bay, Victoria, Australia, and transported to the recirculating sea water system at the Zoology Department of The

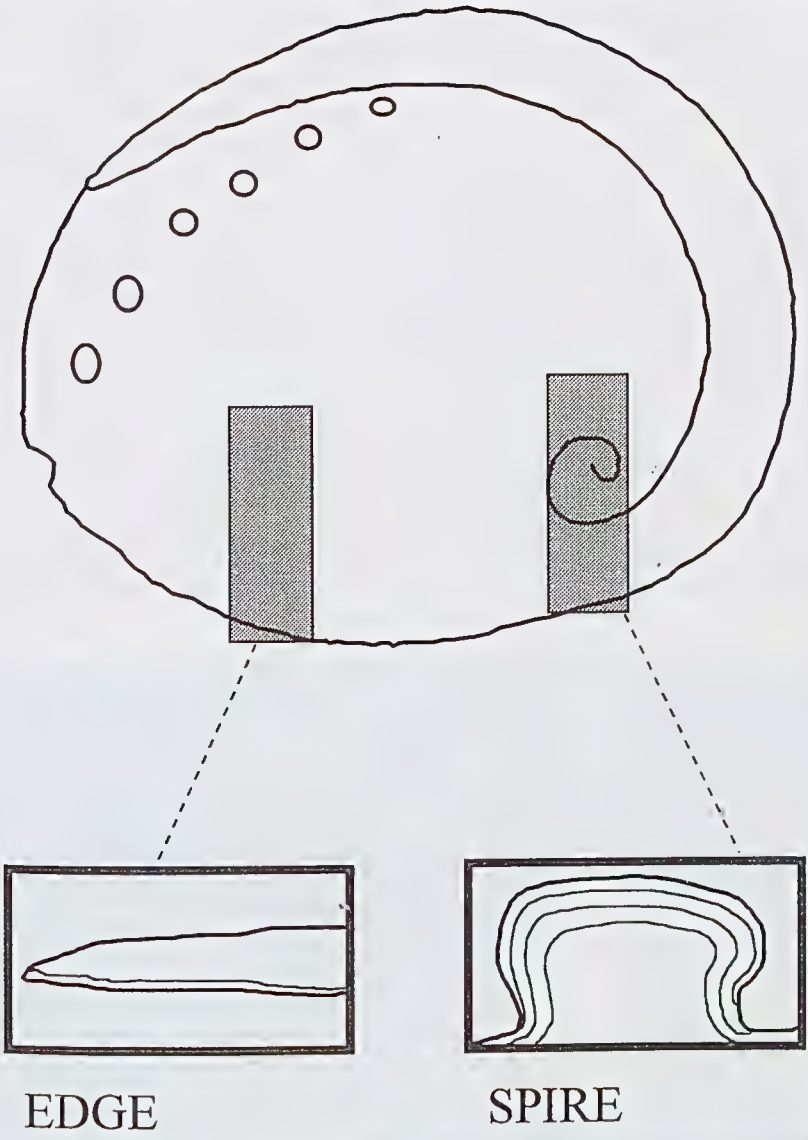

Figure 1. Ventral view of an abalone shell, showing where shell pieces were cut from the edge and spire to examine manganese marks.

University of Melbourne. All abalone were maintained within a temperature range of 17 ± 2 °C during the marking trials. An initial experiment was conducted to determine what concentrations of manganese were lethal to abalone immersed in manganese – sea water solutions, over a 48 h period. Abalone were placed in 20 L aerated buckets of sea water (5 per bucket) to which manganese ion was added (as $MnCl_2.4H_2O$). The amount of manganese added was varied with respect to Mn:Ca ratios, as it is known that increasing ratios of strontium to calcium affect the growth of molluscs (Buchardt and Fritz 1978). Concentrations of 189, 378, 945 and 1890 mg L^{-1} were used, corresponding to Mn:Ca ratios of 1:10, 1:5, 1:2 and 1:1. To observe any adverse effects of manganese treatments, abalone were rinsed and kept in normal sea water with regular feeding and flushing for a period of 10 days.

To determine the manganese concentrations and immersion times needed to produce useable time-marks for age validation, abalone were immersed for 48 or 96 h. All buckets contained 5 adult abalone ranging in length from 80 – 100 mm, with 2 or 3 juvenile abalone of size 50 – 70 mm. Based on the initial experiment, Mn:Ca ratios of 1:200, 1:100, 1:50, 1:20 and 1:10 (9.5 to 189.0 mg L^{-1} of $MnCl_2.4H_2O$) were used. To ensure that incorporation of manganese into the shell had been completed, the abalone were kept in normal sea water, with regular feeding and flushing for a "consolidation" period of 18 days. The abalone were frozen over night, thawed and the body separated from the shell. Pieces were cut from the spire and edge of the shell (Fig. 1), in areas corresponding to previous studies (Erasmus *et al.* 1994; Shepherd *et al.* 1995a, b; Day *et al.* 1995). The spire and edge pieces were embedded in polyester resin and the blocks were cut to reveal cross-sections of the shell. The surface was finely ground using three grades of emery paper and polished on a variable orbital polishing machine. The blocks were then cut to produce uniform 2-mm thick sections for analysis.

Manganese carbonate marks within the abalone shell sections were viewed under a beam of electrons in a vacuum, using optical microscopy, as described in Hawkes *et al.* (1996). Identifiable CL marks were scored for length and thickness (length scores ranged from 0 = no mark to 10 = very long; thickness scores from 1 = very fine to 5 = very broad). This system is based on a previous method to compare fluorochrome marks in shells (Day *et al.*, 1995). The total length included both orange-red (calcite) and yellow-green (aragonite) CL marks within a section (Hawkes *et al.* 1996). The thickness of marks was scored according to the width of the aragonite CL bands only. The

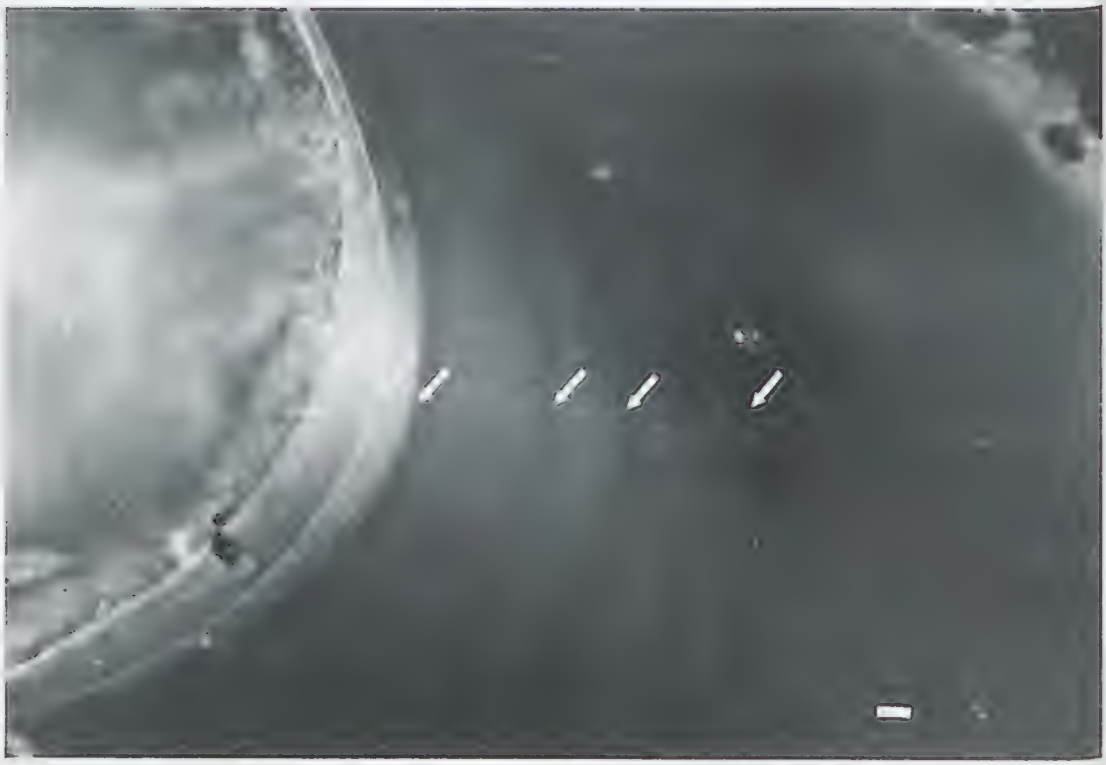

Figure 2. Cross-section of the spire of *Haliotis rubra*, photographed under normal reflected light and cathodoluminescence, showing natural spire layers (arrows), which have been used to age individual abalone, but require validation, inside a fine manganese mark (arrow-asterisk) formed when immersed in 189 mg L^{-1} of $MnCl_2.4H_20$ in sea water for 96 h. Scale bar = 50 mm.

average length and thickness of manganese marks were compared between treatments for both the spire and edge regions. As initial observations showed length and width scores were closely correlated, these scores were incorporated into a total score, where: total score = total length + width/4, in later trials. The scores were subjected to an analysis of covariance, with immersion time as a fixed factor, and manganese concentration as a covariant.

Using abalone collected from the same site, two further experiments were conducted in the same way to investigate the consistency of marking between experiments and to compare marking effectiveness at higher concentrations and longer immersion times (136 – 272 mg L^{-1} at 48 – 144 h).

To show that the marking technique works in the field an experiment was conducted on abalone collected at a depth of 3 m off Rickett's Point, Port Phillip Bay. One hundred and fifteen abalone were collected and stained at a concentration of 200mg L^{-1} over a 48 hr period. After the staining period abalone were kept in the holding pens on the reef for 14 days before they were collected and frozen to analyse the shells. In addition, 10 abalone shells that had not been stained were sectioned to check for natural fluorescent marks under CL.

Results

All abalone immersed in concentrations ≥ 945 mg L^{-1} of $MnCl_2.4H_20$ died within four days after the 48 hour immersion. Abalone at 379 mg L^{-1} appeared weak and stressed, with their body and shell raised off the bucket wall, but recovered quickly once flushed with normal sea water. The extension of tentacles beyond the shell rim, a rapid response to touch and a normal feeding rate indicated the

Table 1. ANOVA analyses of the effects of concentration and immersion time on scores of the length and width of manganese marks observed in edge and spire sections of abalone.

	Degrees of freedom	Mean square	F
Spire Mn length			
Concentration	1	4.929	1.303
Immersion time	1	10.596	2.801
Interaction	1	6.210	1.641
Error	66	3.783	
Spire Mn width			
Concentration	1	0.888	0.690
Immersion time	1	2.329	1.811
Interaction	1	0.000	0.000
Error	66	1.286	
Edge Mn length			
Concentration	1	96.574	16.450*
Immersion time	1	0.782	0.133
Interaction	1	2.478	0.422
Error	62	5.871	
Edge Mn width			
Concentration	1	36.740	25.161*
Immersion time	1	0.169	0.116
Interaction	1	3.577	2.449
Error	63	1.460	
Shell region			
Mn length	1, 138	124.570	24.409*
Mn width	1, 133	15.566	10.372*

abalone immersed at 189 mg L^{-1} of manganese were not stressed. In the latter two concentrations all abalone were alive when the experiment was concluded after 10 days. As it is difficult to measure any other sub-lethal effects, and we wished to ensure that long term deleterious effects were unlikely, manganese concentrations $\leq$ 189 mg L^{-1} were chosen to investigate the effects of varying concentration and length of immersion on the marks produced.

A cross-section of the spire region of a shell, double exposed under visible light and a beam of electrons is shown in Fig. 2. The yellow-green mark of manganese in aragonite is clearly visible within the section. By adjusting the intensity and amount of reflected light and CL, both normal growth layers and manganese induced marks may be observed simultaneously as shown, making this technique suitable for growth increment analysis.

Manganese induced marks within sections of the edge and spire of abalone shells varied in length, thickness and brightness. The mean length and thickness of marks in abalone immersed in 48 and 96 h treatments were plotted against the natural log of the concentrations of manganese in sea water (Fig. 3). Results for the length and thickness of scores were very similar. At the growing edge, the mean length and thickness of marks increased significantly with the concentration of manganese used (Table 1, Fig. 3a, b). Under the spire the maximum mean length and thickness of marks were produced at 38 and 95 mg L^{-1} in both the 48 and 96 h treatments, but no significant effect of concentration could be demonstrated (Table 1, Fig. 3c, d). In either shell region, no significant effect of immersion time on CL scores could be demonstrated (Table 1). CL marks were significantly longer and thicker at the edge than under the spire (Table 1).

To investigate whether marking ability varied between experiments, as found for fluorochromes (Day *et al.* 1995), a new batch of abalone was treated in a concentration of 189 mg L^{-1} for 48 h, as in the previous experiment. A comparison of the length of the marks between these experiments, which were carried out in winter and autumn, shows that edge length scores were higher than spire scores in the experiment conducted in winter, whereas similar scores, between shell regions, were obtained in the autumn trial (Fig. 4). The length of marks at the edge in the initial experiment were significantly ($p = 0.047$) higher than the latter, and vice versa ($p = 0.024$) for marks under the spire.

To investigate manganese marks in abalone subjected to higher concentrations and longer immersion times, abalone were treated to concentrations of 136, 204 and 272 mg L^{-1} of manganese in sea water baths for 48, 96 and 144 hours (2, 4 and 6 days). As the earlier results for length and width were similar, total scores were used. No animals died during the experiment. The variability of the total scores within each treatment is evident in the spread of the box plots in both shell regions (Fig.

Table 2. ANOVA analyses of the effects of concentration and immersion time on total scores of manganese marks observed in the edge and spire sections of abalone.

	Degrees of freedom	Mean square	F
Edge CL total score			
Concentration	2	11.201	1.322
Immersion time	2	243.784	5.169*
Interaction	4	33.394	3.942*
Bucket effect	1	7.803	0.921
Error	80	8.471	
Spire CL total score			
Concentration	2	3.586	0.518
Immersion time	2	0.709	0.102
Interaction	4	10.814	1.562
Bucket effect	1	0.056	0.008
Error	80	6.921	

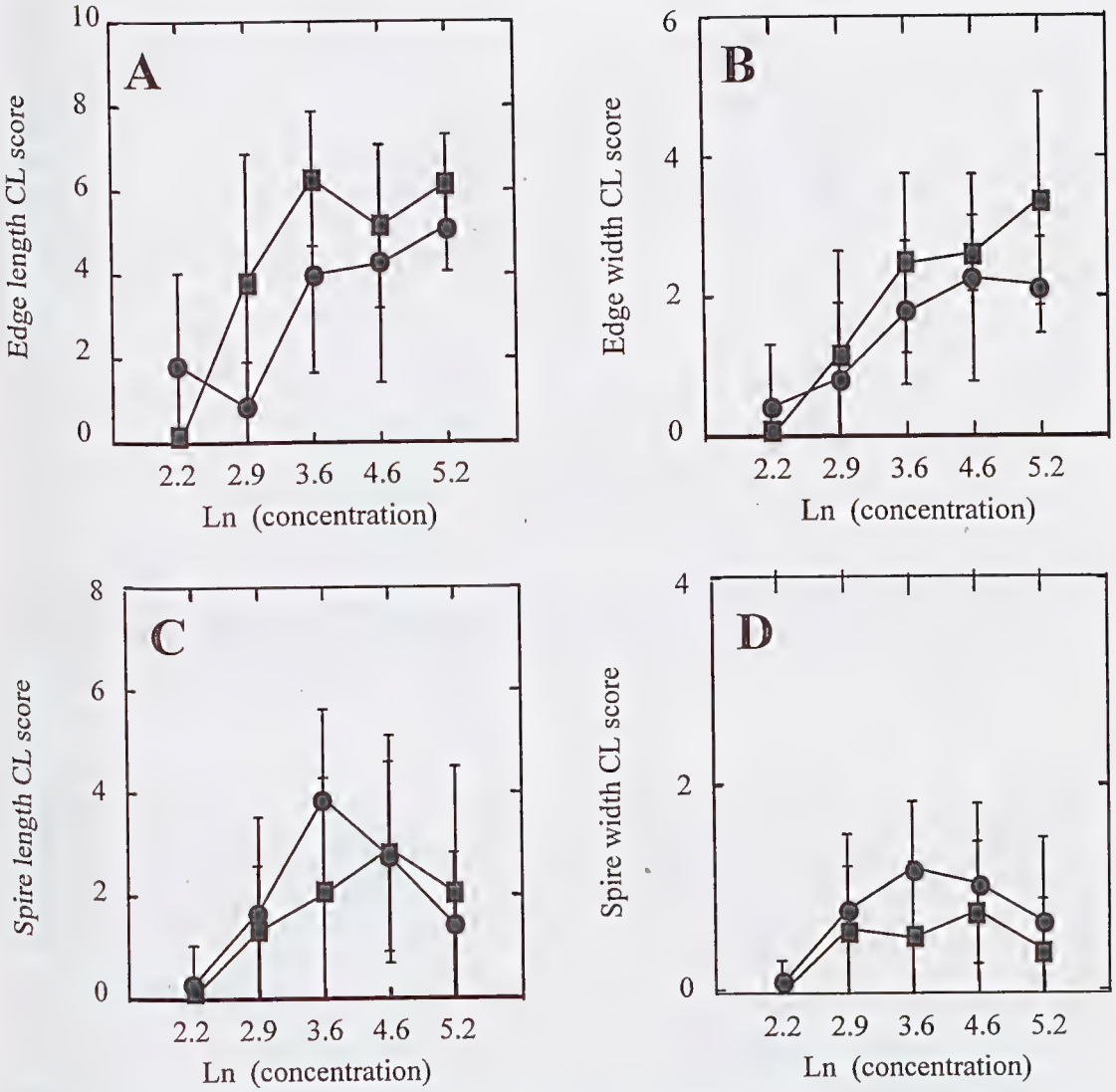

Figure 3. Comparison of scores for mean length (A) and mean width (B) at the edge and mean length (C) and mean width (D) at the spire of manganese marks in shells of abalone immersed in a range of concentrations of 9.5 to 189 mg L^{-1} (natural log) of $MnCL_2.4H_20$ in sea water for 48 h (●) and 96 h (■). Standard error bars are shown.

5). At the edge there was a significant interaction between immersion time and concentration (Table 2). This reflects the fact that scores at the growing edge increased with increasing immersion time, except in the most concentrated treatment (Fig. 5a). This may indicate some toxicity of manganese at this concentration over longer periods. The scores obtained for the spire were lower than the corresponding edge scores. They tended to decrease with concentration, and there was no obvious trend with immersion time (Fig. 5b). However, the highest mean score of 4.71 ± 3.37 was found in the middle range concentration (204 mgL^{-1}) with 144 h immersion.

In the field experiment, 108 abalone of the 115 marked were alive 14 days after the experiment. Clearly visible manganese marks were found in 90 sections of the edge region and 64 sections of the

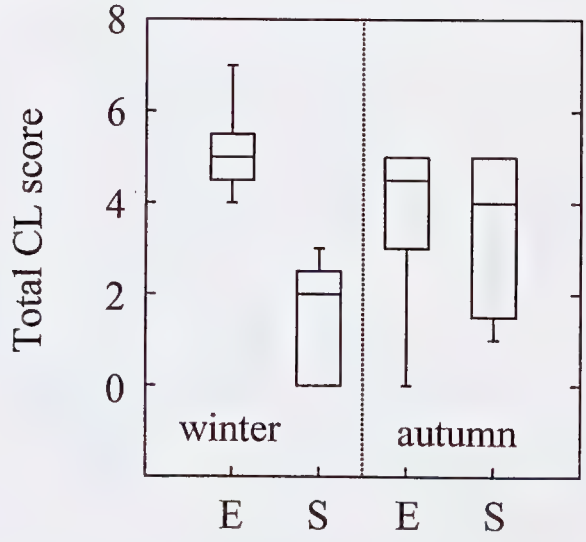

Figure 4. Boxplots of scores of manganese marks from the edge and spire shell region of abalone immersed in 189 mg L^{-1} of $MnCL_2.4H_20$ in sea water for 48 h during experiments conducted in late winter, 1994 and autumn 1995. Sample size = 5 abalone for each treatment. The median, upper and lower quartile and range of the data are shown by each plot.

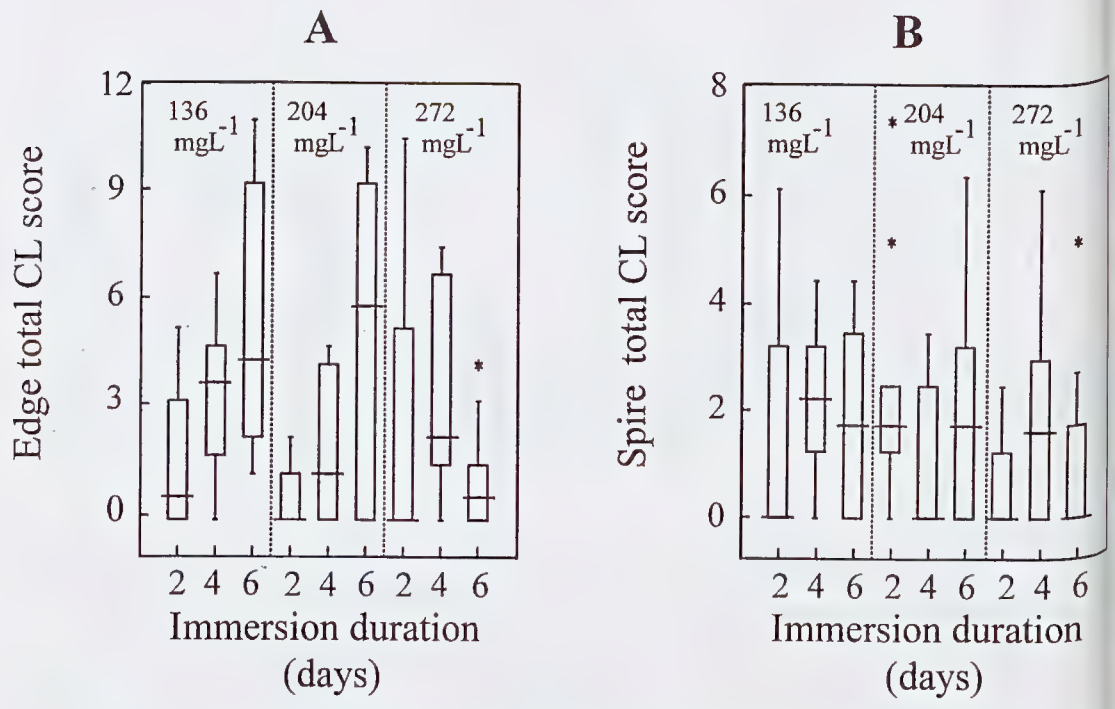

Figure 5. Boxplots of scores of manganese marks from the edge (A) and spire (B) shell region of abalone immersed in 136, 204 and 272 mg L^{-1} of $MnCL_2.4H_20$ in sea water for 48, 96 and 144 h (2, 4 and 6 d). Sample size = 10 abalone for each treatment. The plots are as in Figure 4, but here outliers are presented and denoted by stars.

spire region of these shells.

We observed natural luminescence from calcite and aragonite shell within unstained abalone specimens. In the prismatic layer both orange-red and yellow-green irregular banding was found. The marks observed in the nacre of adult abalone (80 – 100 mm) were mostly hair-like and irregular. However, numerous fine luminescent bands, or rings, were commonly found within the spire of juvenile abalone (30 – 50 mm), and occasional bands were recorded within the edge nacre. This natural CL banding was mostly dull and could be distinguished from the brightly marked layers produced in our staining experiments. However, the colours of natural CL bands where very similar to those of stained layers.

Discussion

Critical levels of manganese

For a marker to be useful it must not induce mortality (Behrens and Mulligan 1990). Manganese appears to be non-lethal except at doses much higher than those required to produce CL marks in shells. Our results suggest manganese chloride concentrations of less than 272 mg L^{-1} should be used to time-mark shells of blacklip abalone. We have obtained over 90% survival in all immersion experiments of blacklip abalone exposed to levels of manganese at or below 272 mg L^{-1}, and the marking results do not suggest any toxic effects except perhaps at 272 mg L^{-1} for 6 days. Pirker and Schiel (1993) found the survival rate of the abalone, *Haliotis iris*, marked with fluorochromes decreased when experiments were conducted in the field. Our *in situ* immersion experiment using manganese, at a concentration of 189 mg L^{-1}, demonstrated the same high survival rate as in the laboratory experiments.

Comparison of manganese marks

Marked shell layers were scored to determine if measurable differences in marking ability using manganese could be detected in abalone exposed to different concentrations and immersion times. In previous abalone marking experiments using fluorochromes Pirker and Schiel (1993) found brighter marks in juvenile abalone immersed over longer time intervals, and Day *et al.* (1995) found the effectiveness of marking at the shell edge increased with time and concentration. However, this was dependent on the fluorochrome used, the season and the batches of abalone used in experiments. Our experiments show no significant difference of CL mark scores between 48 and 96 h immersion in either shell area. There was a trend towards better manganese marks at the shell edge with increasing concentration, but scores were lower at the highest concentrations. Under the spire, concentration had less effect, and marking may reach a maximum at intermediate concentrations.

Calcite and aragonite polymorphs differ in the ease with which cations can be substituted within the crystal matrix (Sommer 1972; Tucker and Wright 1990; Carriker *et al.* 1991), although mineralogical control of trace element composition within mollusc shell appears to vary between species and ontogeny (Carriker *et al.* 1991; Mann 1992). In our study, manganese was only deposited within calcite layers in a short band at the growth margin in the edge sections, so that the length scores depended mostly on nacre deposition behind the growth margin, and width was scored across bands only within nacre. Thus the mineralogical control of manganese deposition would have had little or no effect on overall scores.

Substitution of Ca^{2-} with other elements is subject to physiological control (Mann 1992). Wilbur and Saleuddin (1983) concluded that the rate of ion movement across the mantle epithelia of molluscs was dependent on concentration, but only up to a saturation point. This suggests the rate of substitution of Ca^{2-} with Mn^{2-} across the mantle epithelium at the shell edge may have reached a saturation point at the highest concentrations. At 272 mg L^{-1} the concentration of manganese may induce some physiological stress, leading to less shell calcification and thus less marking. The significantly lower marking scores under the spire are presumably due to differences in deposition

rate, at least in part. The rate of shell deposition appears to be much faster at the growing margin than in the central region in molluscan shells (Wilbur and Saleuddin 1983). Furthermore, the characteristic flattened conical morphology of the abalone shell is a result of rapid deposition of oblique layers extending the outer margin and gradual deposition of horizontal layers thickening the inner regions (de Jong 1990).

The fact that under the spire the highest mean scores were found for intermediate concentrations may indicate that saturation limits within the extrapallial space under the spire are reached more rapidly, as the process of calcification within the inner region is slower, or that transport of manganese via the haemocoel is slow. If higher concentrations induced stress we would have expected this to have affected marking at the edge in the same way. However, perhaps blood flow to the spire region was reduced by higher concentrations.

The rate of shell growth may well be an important factor in determining CL scores in our experiments. The amount of Mn^{2+} deposited will depend on the amount of shell produced over the marking period. The lack of demonstrated effect of immersion time may be an artifact of the staining process, as water flow is necessarily reduced during staining. As abalone activity and growth is markedly affected by water flow the shell deposition process may slow during the experiment, so little marked shell is deposited after 48 h.

The variation within treatments may be large because some abalone were laying down shell rapidly while others were not growing at all. Leighton (1974) and Momma (1980) found variable growth between individual juvenile abalone in the laboratory over short periods, and we have observed growth spurts in juvenile abalone (Day, unpublished data). Field studies on abalone have also shown that the growth of individuals is highly variable both within and between seasons (Shepherd and Hearn 1983; Day and Leorke 1986; Day and Fleming 1992). Abalone are opportunistic feeders which rely on an unpredictable supply of drift algae, and their growth pattern appears to be related to this feeding regime (Day and Fleming 1992). Day and Fleming (1992) showed that growth characteristics may be dependent on previous feeding history, and Day *et al.* (1995) found that better fluorochrome marking was achieved with pre-fed animals, as compared to starved abalone, over a 24 h immersion treatment. However, these studies of growth and marking concern only growth at the edge of the shell. It remains to be seen whether food supply will influence deposition under the spire and therefore the ability to mark the shell for age estimation. Our field trial suggests that with our current methods we can expect to produce clear marks in about 60% of abalone which should be sufficient for validation trials.

Natural luminescent marks

If manganese is to be used to time-mark shell layers it is important that the marked layers can be distinguished from any natural marks. Sommer (1972), Barbin *et al.* (1991 a; b) and Barbin (1992) found that ambient levels of manganese produce natural luminescent growth zones in calcite in bivalves of marine, estuarine and freshwater habitats. The most intense marks were found in freshwater species (Sommer 1972). Yellow-green luminescence within nacreous layers was found only in hyposaline species; shells from marine habitats were non-luminescent (Barbin *et al.* 1991b; Mazzoleni *et al.* 1995). However, Barbin (1992) detected medium to dull CL marks in aragonitic layers of *Nautilus pompilius*, and weak luminescence in *N. macromphalus*. We have detected natural CL bands, similar in colour to our staining marks in abalone, especially juveniles, although natural marks were much duller and narrower than our marks. If these represent manganese induced marks, then the usefulness of manganese as a marker will depend on the local ambient levels of manganese and on the rate of mineralisation of shell during any doping period, as staining marks need to be much brighter than any natural marks.

Manganese marks versus fluorochrome marking

Other hard tissue markers have hitherto successfully stained the outer margin or growing edge region of the abalone shell, but not the spire (Pirker and Schiel 1993; Day *et al.* 1995). Age cannot

be validated by growth lines occurring at the outer margin of the abalone shell as these growth checks are irregular and have been related to disturbance during capture or handling (Day and Fleming 1992). In contrast, manganese is incorporated within both the spire and edge regions. In addition, the length of CL bands at the outer edge were 3 – 4 times greater in magnitude than most fluorochrome bands.

This difference may be related to the movement of the stains across membranes. Fluorochromes are large organic molecules that chelate to $CaCO_3$ crystals, while manganese is incorporated within shell layers by the substitution of Mn^{2-} for Ca^{2-} ion in the formation of carbonate crystals (Sommer 1972). It is expected that the Mn^{2-} ion would be easily transported to sites of calcification since the stoichiometry of these two anions is similar. However, the larger fluorochrome molecules may not move across various membranes as readily, and this may restrict their ability to stain inner areas of calcification. It is possible that fluorochrome and manganese staining of the edge region is caused by the direct absorption of the dye from sea water across the mantle epithelium into the extrapallial fluid as has been postulated for the deposition of calcium and trace elements deposition (Wilbur and Saleuddin 1983; Carriker *et al.* 1991). The formation of luminescent layers under the spire, however, indicates manganese is absorbed across the body wall into the haemocoele from where it is transported to the mantle epithelium throughout the body. In addition, the synthesis of aragonite in gastropods involves crystal formation below many layers of protein sheets (Nakahara 1981) and the larger fluorochrome molecules may not penetrate through these layers as easily as the Mn^{2-} ion.

The fact that manganese is incorporated into layers deposited under the spire, and that it can be detected *in situ* using CL, such that manganese marks and natural growth lines can be seen at the same time, makes this technique useful for age validation studies. Since simultaneous staining of the edge and spire regions occurs, this technique may also be useful in comparing the timing and frequency of shell layers between these areas.

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Feeding effects of postlarval red abalone, *Haliotis rufescens* (Mollusca: Gastropoda) on encrusting coralline algae

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Abstract

Detailed examinations of an algal-microherbivore symbiosis have revealed mutualistic components of such herbivore-plant interactions. High-resolution photomicroscopy and experimental analyses in the field and laboratory were used to evaluate effects of foraging by *Haliotis rufescens* (red abalone) postlarvae ~ 200 μm in length, on their encrusting red algal hosts, *Lithothamnion* (=*Lithothamnium*) *californicum*, *Lithophyllum lichenare*, and *Hildenbrandia rubra* (=*H. prototypus*). We have quantified the microscopic food availability, postlarval foraging behaviour, changes in stomach and faecal contents, growth, and mutualistic effects of grazing. Host algae were found to benefit both from a reduction in coverage by epiphytic algae, and from enhancement of vegetative growth.

Introduction

Planktonic larvae of the gastropod mollusc *Haliotis rufescens* Swainson, 1822 (red abalone) are induced to settle and metamorphose specifically upon contact with various species of encrusting red algae (Morse *et al.* 1979; 1980; Morse and Morse 1984). This substratum-specific recruitment of the larvae is induced upon contact with γ-aminobutyric acid (GABA)-mimetic molecules. These molecules, inducing settlement and metamorphosis, are uniquely available to the larvae at the surfaces of encrusting red algae (Morse and Morse 1984). Juveniles of this and several other species of *Haliotis* are found primarily associated with encrusting red algal substrata (Crofts 1929; Shepherd 1973; Morse *et al.* 1979; 1980; Saito 1981; Douros 1985; Hooker and Morse 1985; Shepherd and Turner 1985; Shepherd and Daume 1996), and the larvae of 13 different species of *Haliotis* have been shown to settle and metamorphose specifically in response to encrusting red algae, extracts of these algae, the purified GABA-mimetic compounds, or GABA (Morse 1984; 1992; 1994).

The symbiotic interaction between *Haliotis* and the recruiting crustose red algae has been suggested to be mutualistic, with reciprocal advantages to both the plant and animal (Morse *et al.* 1979; 1980; Morse and Morse 1984; Morse 1992). The advantages to the animal have been discussed and documented previously (Morse *et al.* 1980; Morse and Morse 1984; Morse 1994). What is more difficult to establish is the possible benefit that might accrue to the host algae. Adey (1973) and Steneck (1982) have shown that a particular encrusting red alga, *Clathromorphum circumscriptum*,

is dependent upon the grazing action of adult *Acmaea testudinalis* limpets, which often settle as larvae and remain associated with their encrusting algal host (Steneck 1982). In the case of *Haliotis*, however, these molluscs initially settling on encrusting red algae typically are mobile, remain in coralline habitats until the abalone are ~ 6 mm in length (Shepherd and Daume 1996), but leave these substrata as they grow. Adult abalone then become more common in rock crevices, where they feed on various foliose algae (cf. Cox 1962; Hooker and Morse 1985). If the association between *Haliotis* and the encrusting red algae is directly advantageous to the algae, such benefits must accrue while the haliotids are young and still associated with their initial algal host. We report here experimental data that support the hypothesis that small *Haliotis rufescens* postlarvae, soon after settling onto crustose red algae, can in fact both decrease epiphytic overgrowth and enhance vegetative growth of their host algae in a mutualistic symbiosis, before the molluscs grow to larger sizes. Such influences of small postlarval organisms in natural communities rarely have been considered.

Materials and Methods

Haliotis rufescens and its encrusting algal substrata were collected from shallow subtidal areas off Santa Barbara, California. Larvae and juveniles were raised in the laboratory as described previously (Morse *et al.* 1979; 1980). Following cultivation of the lecithotrophic larvae for 7–10 d at 15 °C, settlement and metamorphosis on encrusting red algal substrata were induced by exposure of the developmentally competent, swimming larvae to suitable algae-encrusted rocks for 0.5–1 h (Morse *et al.* 1979; 1980). Postlarvae were studied throughout their growth from settlement (at a shell length of ca. 200 µm). Larger juveniles and broodstock of *H. rufescens* were collected from the field.

Small rocks of igneous and metamorphic mineral composition (<250 g each) were collected from tide pools near Santa Barbara, California, where they were common both in tidal pools and subtidally. Rocks were selected that were encrusted primarily with the common coralline red algae *Lithothamnion* (=*Lithothamnium*) *californicum* and *Lithophyllum lichenare*, and the uncalcified encrusting red alga, *Hildenbrandia rubra* (=*prototypus*). Algae were identified according to Abbott and Hollenberg (1969; 1976), with assistance and advice from I. Abbott, G. Hollenberg, and R. Steneck (pers. comm.). Rocks encrusted with these algae are common in shallow subtidal habitats of postlarval *Haliotis rufescens* (Morse *et al.* 1980), and can be used experimentally (Morse and Morse 1984) for studying *H. rufescens* settlement, metamorphosis, and grazing.

Laboratory Observations

At 2–3 d intervals following metamorphosis, at least five groups of abalone on different rocks were observed as described below, and their activities tabulated. Submerged algae and molluscs were viewed clearly by placing a microscope cover slip on the water's surface film. A Zeiss 16-mm objective lens with iris provided a long working distance and depth of field, at 80X–160X on a compound microscope, or at 10X–100X with a 35-mm camera and bellows. A halogen fiber-optic illuminator used at <1/10 maximum output in a darkened room provided cool, focused illumination for observations. A focused, high-speed electronic flash unit (Zeiss) provided photographic illumination at ca. 1/5000 sec.

Field Observations

Quantitative, high-resolution field experiments testing the impact of microscopic *H. rufescens* grazers, begun May 4, 1979, were completed with three matched pairs of algae-encrusted rocks roughly 5 cm in diameter. Pairs were matched as closely as possible for algal patch shapes and sizes. Two pairs (for replication) of the rocks had live encrusting algae; a third pair (without replication here) had encrusting algae killed by boiling ("no live encrusting algae," below). These substrata were suspended from a floating wharf in outer Santa Barbara Marina, with monofilament fishing line (0.5 mm diam.) tied around the center of each rock. These rocks were suspended at a depth of 40 cm,

approximately 15 cm apart. Their order in the row was changed frequently during the experiment to minimize any subtle differences in physical environments. Additional rocks (treated as those described above) for further replication were lost during the experiment, although their less complete sampling yielded additional qualitative results. Later supplementary observations of the rocks remaining 4 mo and 12 mo after the start of the experiment showed that the encrusting algae survived in this environment. Each of the algal species also occurred on adjacent cement floats during the experiments.

In these experiments, larval *H. rufescens* were settled periodically on the algae by placing the rocks in a container of competent larvae (500-ml volume; 40 larvae/ml) for 30 min in the field. Every 4 days for over 2 mo, *H. rufescens* was restocked this way consistently on two of these rocks (from two pairs, each with one rock selected arbitrarily at the beginning of the experiment) while the other four rocks received no molluscs. These four rocks were placed in a container of laboratory seawater while the other two rocks were being restocked in the container with larvae, thus controlling for any effects of the treatment. Swimming animals such as potentially grazing amphipods would have had equal access to each rock, but only twice was an amphipod seen on the rocks during the observations. Each rock used had at least five well-separated patches of *Lithothamnion californicum* on each side; these provided at least five pseudo replicates (or subsamples) on each side for quantitative microscopic analyses. (Statistics therefore compared experimental pairs of rocks, with duplicate rocks for the presence of grazers, rather than attempting to compare rocks in general.)

At two-week intervals, photographs were taken in the field with a 55-mm micro lens for 0.25 X photographs, and with additional extension tubes for 1.5 X photographs. The camera's micrographic reticle focusing-screen enabled viewing a clear image at 1.5 X, for focusing on the center of each rock surface. A 10x13-cm photographic glass plate (stripped of emulsion with hypochlorite bleach) flattened the rippled water in the pan of rocks being photographed; the plate was cemented inside a wooden frame, which served as a float.

The resulting Kodachrome transparencies were scored for algal abundances by viewing them with diffuse light under a dissecting microscope at 20X. Percentage cover of epiphytic algae overlying the encrusting red algal substrata was determined by a point-intercept method. Five microscope fields sampled at random were scored with an ocular micrometer grid to compute cover of epiphytic (filamentous) algae, based on >120 grid points actually intercepting the algae (after Kitting 1980). Similarly, densities of epiphytic algal filaments were counted in five separate 60X views; these defined five fields (each 12 mm^2) as microscopic quadrats on each rock at each sampling time. Evidence for expansion of the encrusting algal substrata was sought in the close-up photographs. Such growth could be assessed clearly on the monofilament line only, as this line extended around each rock and delineated a clean spatial boundary of known age. Expansion of each alga onto a line was scored as percentage encrustation along the length of line that extended over the alga. Data analysis was performed with the use of Sokal and Rohlf (1969), restricting statistical conclusions to the actual rocks in the experiment. Due to small sample sizes remaining throughout the field experiment, direct observations were necessary to strengthen conclusions. Thus, each of these field experiments was supplemented with subsequent laboratory and field observations of postlarvae and freshly collected algae in flowing seawater.

Results

Initial Abundances of Microscopic Epiphytic Algae

Miniature 1 mm x 1 mm quadrats examined in microscope fields at 200X showed sparse epiphytic algae available on freshly collected algae-encrusted rocks; most epiphytic algae were concentrated where coralline algal patches abutted. Naviculoid diatoms were most common, in densities up to 50/mm^2 on live encrusting algae. Cocconeid diatoms reached a maximum of 30/mm^2. Cyanobacteria and green and red filamentous algae were far fewer. Total microscopic algae together with

Table 1. Summary of observations on feeding of postlarval *Haliotis rufescens* on crustose coralline red algae. All material was maintained at 15°C in flowing filtered seawater. Each observation noted is based on at least three (and usually >5) individuals observed in each manner, on freshly collected *Lithothamnion californicum*. Brackets indicate data are unchanged throughout those ≥2 time periods.

	Age Post-Metamorphosis (days)					
Parameter	0	1–6	10	13	20	45
SHELL LENGTH (mm)	0.20	–	0.25	–	0.50	1.0
MOBILITY	[	10 shell lengths per 37±11 sec	]	<100 shell lengths per day (net)		sedentary
RADULAR MOTION	initially in sac	[smooth sweeping	]	[forceful scraping		]
MEAN NUMBER OF FEEDING MOTIONS (per sec) (intermittant)	[	0.63 – 0.80 (continuous)	]	0.80 – 1.3 [(irregular)	0.80 – 1.1	1.3]
RADULAR WEAR	[	none	]	slight on 2 rows of teeth	on 2 rows	on 4 rows
STOMACH CONTENTS (whole mounts of postlarvae)	velum?	[no solids	]	cyanobacteria plus diatoms		coralline algal cells, cyano-bacteria, diatoms
FECAL CONTENTS	[	no solids	]	$CaCO_3$ fragments smaller than algal cells (<5 μm diam)		$CaCO_3$ fragments larger than algal cells (>5 μm diam)

cyanobacteria represented <2% cover, as sampled with point intercepts with an ocular micrometer grid. Even fewer visible epithallial flakes from the coralline algae were seen, but thin, clear algal mucus was observed to be widespread.

Early Post-Settlement Feeding Behavior

Table 1 summarizes our observations of feeding behavior of the smallest *H. rufescens*. Immediately after we allowed the larvae to settle and metamorphose on each of the encrusting algal substrata, these smallest postlarvae moved their shells and mouths very actively, but cellular solids were not eaten detectably for the first 10 days. During this period, juveniles were very active as their shell length increased from about 200 μm to 250 μm (Fig. 1A). During the first 10 days following metamorphosis, postlarvae traveled about 0.3 shell lengths/sec. The mouth glided quickly, smoothly, and almost continuously over the encrusting algal surface. Some of the mucus on the algae (cf. Morse *et al.* 1980), detectable via adhering microscopic debris, was swept toward the mouth during this activity.

At 10 d post-metamorphosis, ten individuals timed for the interval required for 20 consecutive mouth motions swept their mouths over the algae at an average rate of 0.63 ± 0.21 (S.D.) times per sec. No visible solids appeared in the feces or stomach, viewed in whole mounts of the animals at 1000X. Any bacteria in the stomach or on available algae were too sparse to be detected; bacteria were visible only in additional preparations of damaged, moribund postlarvae, possibly infected or beginning to decompose. Fresh preparations, pressed to expose the radula, revealed at least four rows of well-formed teeth on the radula, with no observable wear even on the oldest teeth (after Kitting 1980) viewed at 1000X (Table 1).

At 12–15 days post-metamorphosis, juvenile feeding became more intermittent, forceful, and less smooth over the algae (Table 1). Ten timings of 20 consecutive mouth motions showed an average of 1.04 ± 0.16 (S.D.) motions/sec. Several intact but damaged (without normal pigmentation) cyanobacteria, small diatoms (~5 μm long), and fragments apparently derived from the coralline algae were found in the stomachs and in fecal pellets (10 x 50 μm; e.g., Fig. 1B) viewed at 1000X. Radulae showed two noticeably worn lateral teeth of the six well developed ones (Fig. 1C). This feeding by distinct scraping continued at least until 60 days following metamorphosis.

Continuing observations of *H. rufescens* juveniles as they grew showed that they became largely sedentary after 20–45 days post-metamorphosis, when the juveniles had reached a shell length of almost 1 mm. By this time, whole cells from the encrusting red algae appeared in stomachs (viewed in whole mounts), and at least four rows of radular teeth showed wear when examined as above.

Effects of Populations of Small Grazers on the Growth of Epiphytic Algae

Each group of *H. rufescens* introduced to the suspended rocks disappeared after 4–8 days; postlarvae were observed (in pans in the field) to be dislodged from occasionally swaying substrata. Furthermore, those larvae and postlarvae among dense erect red algae (e.g. *Acrochaetium*) on such rocks often were consumed by associated terebellid polychaete worms. Restocking of the molluscs every 4 days on the suspended rocks thus maintained postlarvae <8 days old, with almost all <4 days old. Counts of postlarvae before and after each restocking showed an overall population density of roughly 7 individuals/cm^2.

Frequent direct observations, and photographs of experimental and control rocks at two-week intervals, showed noticeable differences in epiphytic algal growth between these rocks with postlarvae and those without. Filamentous epiphytes appeared gradually over the rocks, especially those without postlarvae (Figs. 2A,B,C), although all rocks initially had appeared relatively clean and smooth. The epiphytic cover consisted primarily of a filamentous green alga, *Enteromorpha flexuosa*. Microscopic analyses in the field revealed that after seven weeks, the numerous patches of the encrusting coralline alga *Lithothamnion californicum* on the two rocks with abalone, had less than half the density of filamentous algae covering the *L. californicum* on each matched rock without postlarvae (Fig. 3A; cf. Figs. 2A,B). Pairs of rocks varied somewhat in crustose algal patchiness, and resulting epiphyte

Figure 1. Feeding by postlarval *H. rufescens*. A. Anterior view of an individual 4 days post-metamorphosis on a coralline alga, with 3-celled green algal filament to right and a bryozoan zooid to left. Photographed area is 500 μm long. B. Dorsal view of 25-day post-metamorphic individual with fecal pellets on coralline algal substratum. Shell length ca. 600 μm. C. Compound microscope view of the radula of a 12-day post-metamorphic individual, in a whole-mount of the animal. Distal working end is lowest, with the lowest pair of the six discernible lateral teeth noticeably rounded from wear. (Long slender marginal teeth are less discernible.) Unidentified fragment is visible at upper right on the radula. Radular width ca. 50 μm.

filaments extending from adjacent patches of rock. However, considering mere pseudoreplication of algal patches on each rock, and only two grazer replications remaining throughout the field experiment, the overall density of epiphytic algal filaments on this coralline alga (specifically on these rocks) indicated a statistically significant difference in the presence and absence of the postlarval molluscs (one-way ANOVA; $F = 26.7$, $df_1 = 1$, $df_2 = 2$, $P < 0.05$). The difference in percentage cover of filamentous algae on coralline algae (Fig. 3B) also became noticeable after about five weeks, and was statistically significant (G-test on all raw data, not percentages, after five weeks; $G = 72.4$, 1df, $P < 0.005$).

Coralline algae themselves also appeared to deter colonization by epiphytic algae on these rocks. Coralline algae on rocks without abalone had less than one-fifth the density of algal filaments observed on the rocks having no live encrusting algae or postlarvae (Figs. 3A,B; cf. Fig. 2C); these differences appeared highly significant statistically after seven weeks for both filament counts and percentage cover (ANOVA and G-test, respectively; $P < 0.005$).

The numerous patches of the encrusting uncalcified alga *Hildenbrandia rubra* on the two rocks with abalone also had much lower percentage cover of filamentous algae after about five weeks, compared with the *H. rubra* on the pair of rocks without postlarvae (Fig. 3C; G-test on raw data; $P < 0.005$). In each statistical comparison itself, minimal replication limits generalization, but supplementary qualitative observations often were more conclusive, and consistent with the quantitative data. The dark color of the *H. rubra* (not calcified) prevented reliable counts of individual epiphytic filaments overlying this species, but the epiphytes also appeared to be more dense on the ungrazed *H. rubra*, as they were on the *L. californicum* quantified above. Although the second coralline alga, *Lithophyllum lichenare*, was not sufficiently abundant in our samples to allow quantification of its epiphytes, subjective observations suggested that it, too, had fewer epiphytes when grazed by the postlarval gastropods than when ungrazed.

Effects of Populations of Small Grazers on Coralline Algal Growth

It was necessary for these studies to obtain an index of encrusting algal fitness that might reflect effects of grazing or epiphytism. No significant changes in the density of conceptacles (encrusting algal reproductive structures) nor overall sizes of the crusts were observed during the course of our experiments. However, the systematic close-up photographs revealed clearly quantifiable and significant differences in the vegetative growth of these coralline algae over the introduced surfaces of monofilament line (cf. Fig. 2). This growth clearly was noticeable only in the presence of the postlarval *H. rufescens* (Tables 2 and 3). The monofilament line remained secure around each rock, presenting an inert boundary over which algal growth could readily be detected accurately. Quite consistent results were observed (Table 2); after the third week of the experiment, the grazed

Table 2. Effects of postlarval grazing on the vegetative growth of coralline red algae. Summary of observations, taken by point-intercept method (from 1.5-X photographs), for coralline algal growth over monofilament line, with and without postlarval *H. rufescens* during weeks 3–6. Statistical significance (among subsamples and minimal replication) is presented in the text.

Alga	*H. rufescens* present	Portion of algal monofilament transect overgrown (points overgrown/ total points scored)	(percentage)
Lithothamnion	Yes	24 / 60	40%
"	No	0 / 84	0%
Lithophyllum	Yes	29 / 65	45%
"	No	0 / 20	0%

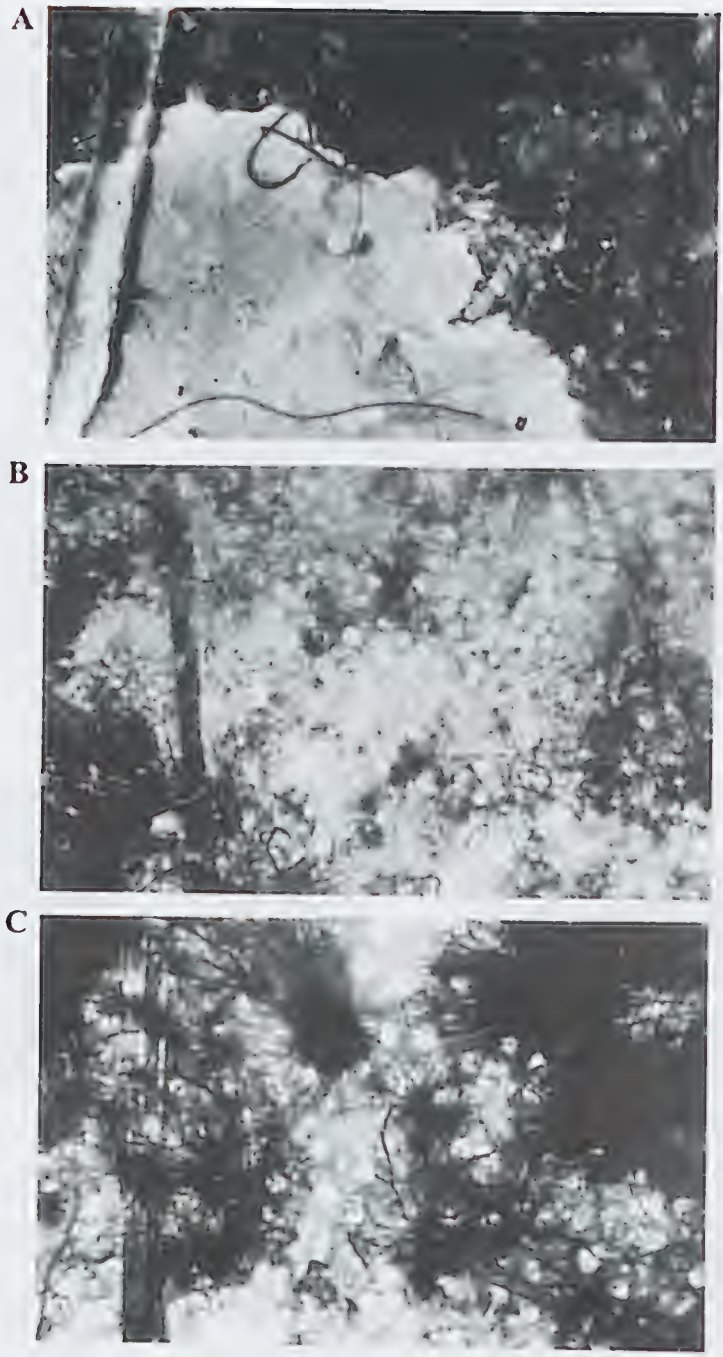

Figure 2. Effects of postlarval grazers on crustose red algae, after 4 weeks of the experimental regime. Each representative photographed area is ~5 mm high (cropped from 1.5X photographic samples). Monofilament line extends over algae near the margin of each photograph. Filamentous green algae overgrow scattered areas. A. Live encrusting algae with average density of *H. rufescens*. Five oblong individuals (<4 d post-metamorphosis) are visible. Coralline algal growth over line is beginning. Two weeks later, coralline growth over the line extended all along each side of the line. B. Corresponding sample of rock without molluscs. C. No live encrusting algae nor molluscs.

Table 3. Effects of postlarval grazing on the vegetative growth of coralline red algae. Summary of observations, for *Lithothamnion* and *Lithophyllum*, by algal patch. Other details as in Table 2 and Methods. G-test on raw data (among patches) indicates statistically significant differences among these rocks, at $P < 0.01$.

Postlarval *H. rufescens* present	Coralline algal patches overgrowing monofilament (patches overgrowing line/ total patches)	(percentage)
Yes	15 / 23	65%
No	0 / 33	0%

Lithothamnion californicum was overgrowing the monofilament line along 40% of the line overlying this crust (measured by the point-intercept method); grazed *Lithophyllum lichenare* similarly showed 45% overgrowth of the line. In marked contrast, the coralline algae on ungrazed rocks showed no detectable overgrowth of the line in any of the possible instances observed (Table 2). For each of these two algae, the differences observed in such growth in the grazed samples versus ungrazed controls were statistically significant (three G-tests on raw data; $P < 0.01$).

A similarly significant effect of postlarval *H. rufescens* is seen in the percentage of coralline algal patches showing enhanced vegetative overgrowth (Table 3). However, neither grazed nor ungrazed *Hildenbrandia rubra*, which had a slower growth rate than the coralline algal species studied, ever was seen to grow over the monofilament line in any of the >20 instances observed in these experiments.

Discussion

Postlarval Feeding on Encrusting Algae

Our detailed analyses of encrusting algae show small-scale but detectable effects of postlarval grazing; young gastropods recruited onto the encrusting algae evidently can help decrease epiphytic overgrowth and increase coralline host algal growth soon after the gastropods settle. At least under relatively calm, shallow-water conditions, even the youngest molluscs settling there (<4–8 days post-metamorphosis) appeared to influence the algal surface, as seen in our quantitative experiments in the field, and in our qualitative observations in the laboratory. The average experimental density of about 7 individuals/cm^2 is probably higher than the densities of juvenile *Haliotis* spp. usually occurring in the natural environment. Morse *et al.* (1980) found overall densities of larger individuals of *Haliotis* spp. (1–22 mm long) to be over 2.2 individuals/100 cm^2 of bottom; they were concentrated on patches of encrusting algae. Our experimental density may approach naturally occurring densities of total postlarval grazers (of several species) settling on such algal patches. Larvae of a number of other grazing species of chitons (Barnes and Gonor 1973; Morse *et al.* 1979) and gastropods (*e.g.*, Heslinga 1981; Steneck 1982) have been observed to settle and metamorphose preferentially on encrusting red algae. Juveniles of a number of species of *Haliotis* (e.g., Crofts 1929; Shepherd 1973; Saito 1981; Morse 1984; Hooker and Morse 1985), limpets (Morse 1980; Steneck 1982), starfish (Barker 1977), and other invertebrate grazers (see Morse *et al.* 1980) also occur preferentially on these encrusting algae; hence, postlarval foraging activity on these encrusting algal substrata may be high. Even though postlarvae may not initially graze host or epiphytic algal cells directly, their active shell movements and disturbance to surface algal mucus appeared to decrease epiphytic algal buildup. Our analyses of microscopic quadrats showed that these algal surfaces possess, in addition to algal mucus, sparse and patchy microscopic algae and epithallial coralline algal fragments, which can be consumed by larger juvenile grazers such as *H. rufescens* >10 days after metamorphosis.

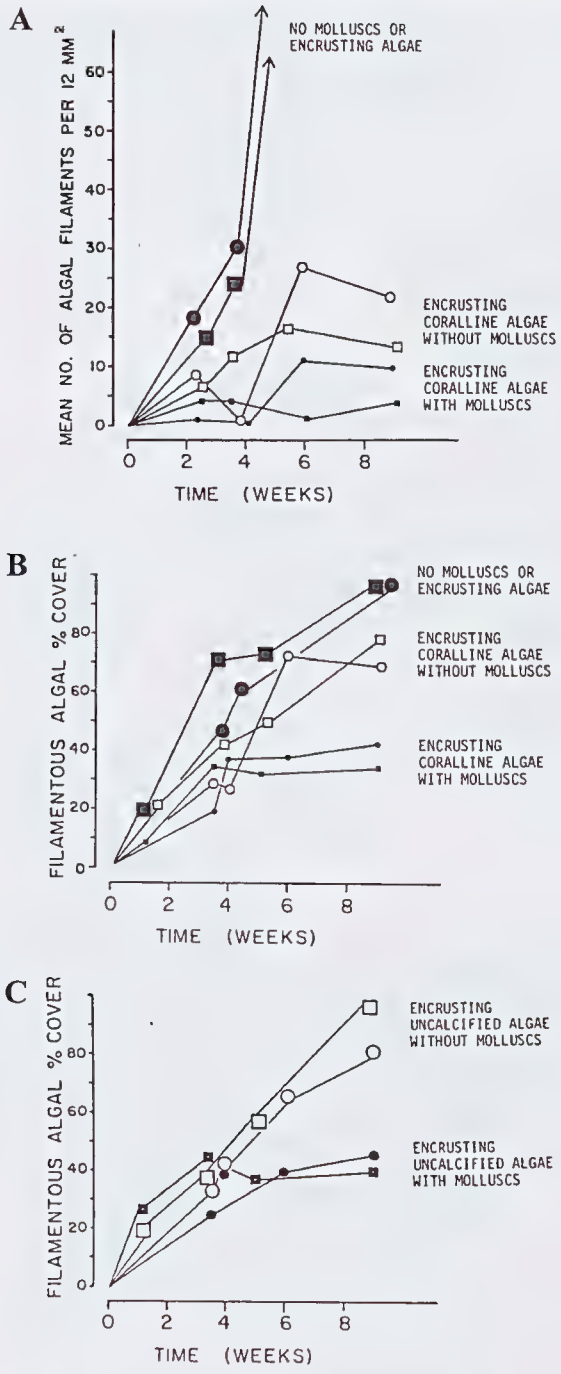

Figure 3. Quantification of epiphytic growth on encrusting algae on each side of experimental rocks. More than five replicate patches of *Lithothamnion* (in Fig. A and B) and *Hildenbrandia* (in Fig. C) were present on each side of a rock. Squares = one matched pair of sides, circle = other matched pair. Open versus closed symbols represent different treatments (as labeled on Figure). Fig. A includes bars as ±1 standard error (among algal patches as subsamples). Data are scored by point-intercept method, as described in Methods. Symbols on Figs. B and C approximate the size of error bars (*i.e.* ± <5%, again among algal patches as subsamples).

The youngest *H. rufescens* demonstrated active foraging behavior with very rapid mouth movement, yet without any detectable particles appearing in their stomachs for the first 10 days of postlarval growth. The rapid, smooth movements of the mouth parts, lack of visible radular wear, and lack of feces all suggest that little scraping occurs in this early feeding stage, but that the youngest juveniles may instead be deriving nourishment from the glycoprotein mucus exuded from the encrusting algal substratum (Morse *et al.* 1980). Associated microorganisms then may become important quickly, since very young abalone in culture grow more quickly on certain unicellular foods than on clean coralline algal surfaces (Hooker and Morse 1985). Young *H. rufescens* also can survive and grow for at least one week following metamorphosis, nourished only by endogenous reserves of yolk and occasionally by ingestion of yolk-rich cells of the velum (the larval swimming organ shed at metamorphosis; Morse 1984; Hooker and Morse 1985; cf. Fretter 1972). Thus, little if any damage to the encrusting host alga need result from the initial colonization by the postlarval *H. rufescens* recruits.

Coralline *Lithothamnion* and *Lithophyllum*, and the tough uncalcified *Hildenbrandia* tissues all were rasped extensively by juvenile *H. rufescens* several weeks old. Adey (1973), Steneck (1982), and Kitting (1984a) found that other gastropods, as small adults, rasped encrusting coralline and tough uncalcified algal species extensively. Future close observations may show that seemingly tough encrusting algae often are browsed by relatively inconspicuous rasping by various small animals.

Mutualistic Symbiosis

Results of the experiments presented here indicate that the symbiotic relationship between encrusting red algae and their associated small molluscan grazers can, in fact, be mutually beneficial to the plant and animal partners. In the case of *H. rufescens*, such mutualistic benefits can be detected during the early period in which the larvae settle and metamorphose, and postlarvae begin to grow on their host algae. As discussed previously, this association benefits the recruited *Haliotis* by providing specifically required morphogenetic inducers that are uniquely available at the surfaces of encrusting red algae (Morse and Morse 1984), and by provision of suitable physical environments, sources of nutrition, and camouflaging pigmentation (Morse *et al.* 1979, 1980; Morse and Morse 1984). Steneck (1982) pointed out the suitability of relatively smooth, extensive coralline algal surfaces for firm attachment by small molluscs such as limpets, and Littler and Littler (1984) showed that another *Lithophyllum* sp. can indicate locations that experience constant immersion and little physical disruption. In addition, our supplementary observations of postlarve placed on freshly collected rocks show that encrusting algae provide a partial refuge from small polychaete worms and other minute predators, which are more concentrated in sediments or foliose and filamentous algae than on the encrusting red algae. A similar refuge effect has been observed for newly settled larvae of *Astraea undosa* (Markell and Morse, in prep.), and for various sessile invertebrates excluded from patches of filamentous algae by herbivorous fishes (Day 1983). However, when numerous large grazers occur on encrusting coralline algae (see Steneck 1983), the refuge afforded by these algae may be reduced.

The encrusting algal hosts appear to derive reciprocal benefits from *H. rufescens* postlarvae (cf. Morse *et al.* 1980; Morse and Morse 1984). Our observations show that these juvenile molluscs can significantly decrease epiphytic overgrowth of the encrusting algae (Figs. 2, 3), and dramatically increase coralline algal growth onto an open (monofilament) surface, at least under the conditions described (Tables 2, 3). These findings provide evidence that the postlarval molluscs can confer benefits to the encrusting algae, particularly when larval recruitment is high (yielding ~ 7postlarvae / cm^2).

Likely detriments to the host alga due to epiphytic overgrowth include accumulation of entrapped sediment and detritus, shading when light is dim, reduction in nutrient and gas exchanges, and suppression of growth by potentially allelopathic epiphytes (after Fletcher 1975). Larger grazers have been reported to remove competing algae and augment the growth of various encrusting algae (e.g. Paine and Vadas 1969; Adey 1973; Vine 1974; Wanders 1977; Slocum 1980). However, epiphytes

do not in general kill encrusting coralline algae in instances where light and nutrients are sufficient underneath the epiphytic cover (Vance 1979) nor when coralline algae slough off enough tissue (Johnson and Mann 1986) or have an overlying canopy of algae (see Figueiredo *et al.* 1996).

Adey (1973) and Steneck (1982; 1983) showed that grazing by larger gastropods can benefit a specific encrusting alga by scraping the alga free of dead epithallial tissue and epiphytic overgrowth. Our supplementary field observations suggest that larger individuals of *H. rufescens* do not decrease epiphytic overgrowth of the host alga noticeably; juveniles 1–3 cm in shell length became sedentary in depressions and maintained a clearing free of epiphytes only about the size of their shell (Kitting, unpublished observations).

Our experiment comparing epiphytic overgrowth on live encrusting algae to that on dead algal surfaces (cf. Figs. 3A,B), and analogous larger-scale experiments (Breitburg 1984) show that these encrusting algae resist epiphytic fouling rather effectively in nature, even in the absence of noticeable grazing by macroscopic herbivores (see Figueiredo *et al.* 1996). One mechanism appears to be the sloughing of algal surface tissues, dislodging epiphytes (Giraud and Cabioch 1976; Adey 1973; Johnson and Mann 1986; Keats *et al.* 1994). Another mechanism might involve the action of common microscopic grazers; such small grazers as microscopic postlarvae are not excluded by cages, nor quantified in most grazing experiments. Such microscopic juvenile invertebrates often might be sufficiently common to help keep encrusting algae clean, even where these algae (upon superficial examination) appear to be ungrazed. This suggested significance of microscopic events on encrusting algae contrasts with previous descriptions of encrusting (coralline) algae as simply "primary space" suitable for colonization by other sessile organisms (e.g. Dayton 1971) or "barren grounds" (e.g. Lawrence 1975) within assemblages of larger organisms.

Mechanisms by which postlarval *H. rufescens* grazers contribute to enhanced growth of the coralline algal hosts (Tables 2, 3) are not yet clear. The extension of the grazed algae over adjacent bare (monofilament) surfaces clearly is vegetative growth. No effect on sexual reproduction, or on the microscopic appearance of the algal conceptacles, could be observed. Possible transport of algal cells or propagules by the browsing molluscs (after Kitting 1980; 1984b on larger gastropods) also might have enhanced encrusting algal accumulation, although a direct test of this possibility was negative with young *H. rufescens* (Hooker and Morse, unpublished observations). Fletcher (1975) has reported experimental evidence demonstrating that filamentous algal epiphytes can suppress the growth of encrusting red algae by allelopathic (chemical) means. It thus is possible that the action of small molluscan grazers can enhance growth of their crustose algal hosts (cf. Tables 2, 3, and Fig. 2) by reducing allelopathic suppression by epiphytes.

A model by Roughgarden (1975) demonstrates how commensalism could evolve into mutualism through increased fitness of colonists, which otherwise could evolve to avoid the symbiosis. We suggest additional factors which may help account for reportedly numerous examples of mutualism. Examples of host organisms evolving to rely partially on (possibly diverse) symbiont colonists (e.g. for removal of epiphytes; cf. Adey 1973), as evident for new colonists (postlarvae) in the present study, suggests the importance of evolution of the host, in contrast to the emphasis on the symbiotic colonist in previous models of the evolution of mutualism (Slatkin and Maynard Smith 1979). These various encrusting red algal hosts may benefit from the settlement of various grazing symbionts while resisting damage from these symbionts. We hypothesize that mutualism often may evolve from adaptations of host species to benefit from the presence of otherwise commensal symbionts.

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The shell microstructure and chronology of the abalone *Haliotis corrugata*

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Abstract

The abalone *Haliotis corrugata* deposits fine and dark rings in the shell near the spire and the dark rings can be used to age the shell. Each dark ring has two distinct microstructural elements; a layer of prismatic structure, possibly calcite, and a juxtaposed narrower layer of granular structure. The crystalline ultrastructure of aragonite between the rings shows seasonal changes in thickness of laminae apparently correlated with seasonal temperature changes. Examination of shells grown in culture showed that three fine rings are deposited during the first year, the first dark ring at an age of about 20 months, and the second between 2 and 2 ½ years; thereafter a dark ring is deposited annually. This aging technique was validated up to about 12 years of age by using growth checks laid down in the shell in about October 1991 during the El Niño as a time-stamp from which growth rates and age could be estimated. Using the aging technique the von Bertalanffy growth equation was fitted to age-length data from La Natividad, Baja California and the following parameters obtained: $K = 0.33$; $L_{\infty}=160$ mm. Catch curve analyses of commercial catch data gave $Z = 0.28$ for a sample in the late 1970s, and 0.66 for one in 1995.

Keywords: *Haliotis corrugata*; rings; shell-aging; shell ultrastructure; El Niño, growth check.

Introduction

The ability to age an abalone shell is valuable for fishery management because growth rates and mortality can be readily estimated without long and costly field experiments (Day & Fleming 1992). The abalone *Haliotis corrugata* Gray is a substantial component of the commercial abalone fishery in Mexico (Guzmán del Próo 1992), but its abundance has declined seriously in recent years possibly through overfishing. The biology of the species is poorly known, so there is a need for basic information on growth and mortality rates for better management of the fishery.

Following the discovery of rings in abalone shells by Hayashi (1955), Muñoz-Lopez (1976) first suggested that the shell of *H. corrugata* could be aged by counting the rings in the spire, but did not validate the technique. In recent studies of the shell of *H. fulgens*, Shepherd *et al.* (1995a) and Shepherd and Turrubiates (1997) have shown that prismatic layers (called rings), possibly calcitic (but see Hawkes *et al.* 1997) and deposited alternately with layers of aragonite in the spire, are a reliable indicator of age, although the picture is complicated by the presence of pigmented and non-pigmented rings. In this paper we examine the pattern of ring deposition in shells of *H. corrugata*. First, we describe salient features of the micro- and ultra-structure of the shell, and then examine the frequency of ring deposition in shells grown in culture. Lastly we apply the technique to samples of

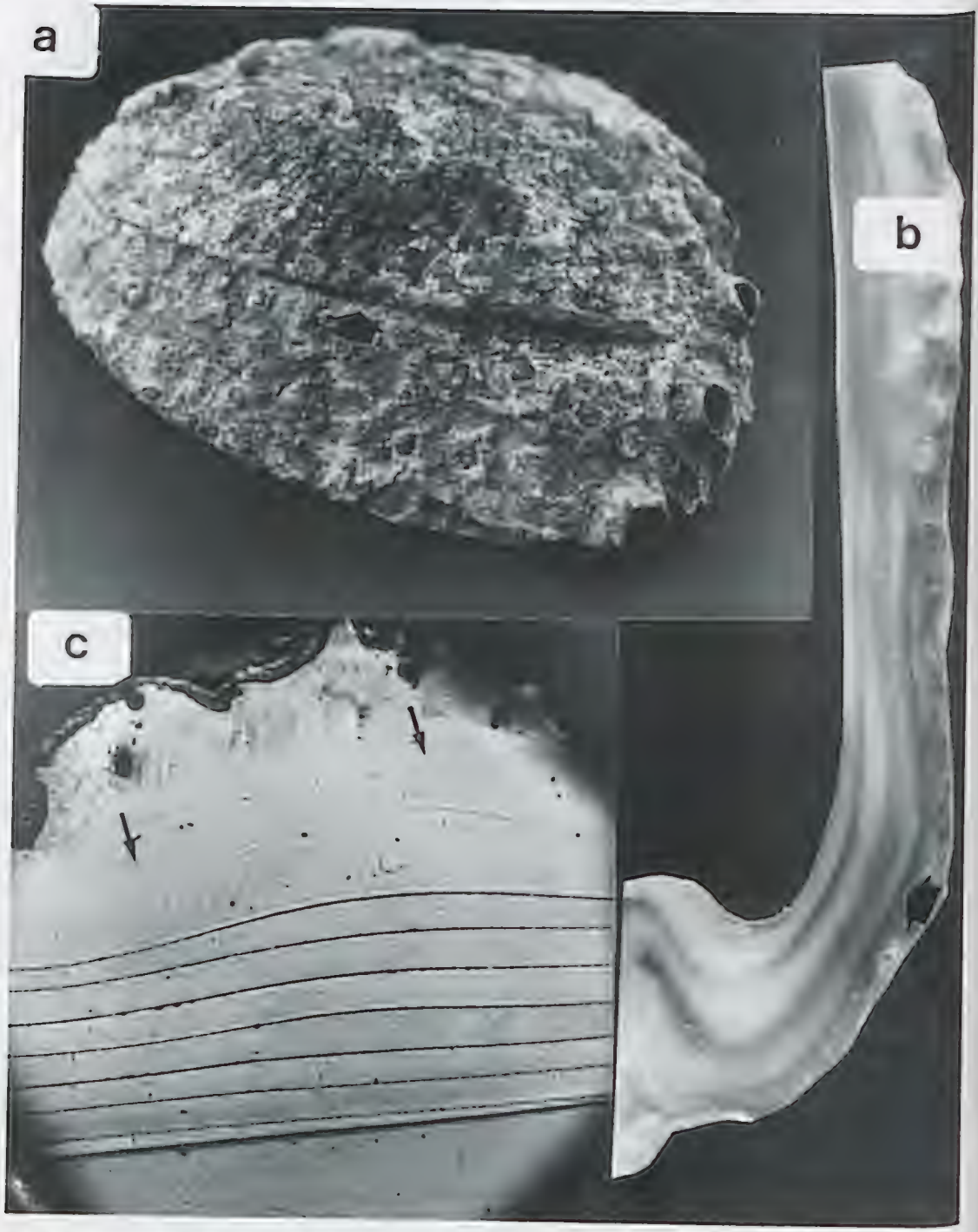

Figure1. a – photo of *H. corrugata* showing (arrowhead) prominent El Niño growth check; b – vertical section at the spire showing (arrowhead) the recommended area in which to count dark rings; c – micrograph of vertical section in the recommended area showing dark rings and (arrowheads) indistinct fine rings.

shells from La Natividad Island, Baja California (Lat.27°51'26"N; 115°07'30"W) and estimate growth and mortality rates.

The strong El Niño event of 1991–2 (Hayward *et al.* 1994) elevated sea temperatures some 2–3°C in central Baja California causing the disappearance of the kelp forests of *Macrocystis pyrifera* from about October 1991 until the end of the next year (J Turrubiates pers. comm.). El Niños can produce conspicuous growth checks in abalone shells (Cox 1962) and did so in 1991 in *H. fulgens* (Shepherd and Turrubiates 1997) and *H. corrugata*. So we used this event as a time stamp to validate the rate of deposition of rings in *H. corrugata*.

Materials and methods

A sample of 50 shells of *H. corrugata*, 12–71 mm shell length (SL) grown in culture in California (McCormick 1992) was examined. The mean growth rate of the shells was 14.3 mm per year (T. McCormick pers. comm.) and was linear with length over the size range examined, so the age of each shell was estimated from length. In addition we examined 224 shells from the commercial catch taken at La Natividad in March 1995, and 214 shells 60–177 mm SL taken during research surveys there from 1990–5.

The spires of the shells were ground with an electric grinder until a minute hole appeared, and then polished with 200 to 600 grit abrasives. These horizontal sections revealed a series of concentric rings. The number of rings was counted under a stereo microscope. Vertical sections of a sub-sample were cut at the spire, polished, set in resin, polished again with fine alumina powder, and etched with dilute HCl as described by Shepherd *et al.* (1995a). The variation in crystalline ultrastructure was examined across the inner nacreous layer of vertical sections by taking a series of micrographs (x5000) under the scanning electron microscope (SEM). The thickness of the laminae in the nacreous layers was then measured from the micrographs in a transect across the section from the outer to inner margin. Because of the difficulty of measuring individually laminae with fuzzy margins we measured groups of 10 and then calculated the mean width of individuals. In all 24 vertical sections were examined in detail, and the rings of 438 shells were examined in horizontal section.

Shells with "El Niño" growth checks (Fig.1a) were examined for growth in two ways. First, we measured shell length to the growth check (SL_1) and the total length (SL). Assuming (a) a uniform birth date on 1 November in accordance with current knowledge of peak spawning in *H. corrugata* (Ortiz *et al.* 1990), and (b) deposition of growth checks on 1 November 1991 (see results), we calculated and then plotted the mean annual increment $(SL-SL_1)/y$ vs the mean length, where y is the period in years from the El Niño date (1 Nov.1991) until the date of capture. This is the classical Gulland-Holt plot (Gulland 1983). A least squares regression analysis gives estimates of the growth parameters of the von Bertalanffy growth curve.

Next, we selected shells taken in March 1995 with El Niño growth checks such that $SL_1 < 140$ mm SL and estimated their age to SL_1 using mean age-length data derived from the growth analyses of the shells taken in March 1995. We added 3.3 years, the period from 1 November 1991 to March 1995, to give an estimated age of the shell, and compared the observed number of rings with the estimated age. The parameters of the von Bertalanffy growth equation were calculated for the age-length data by using a Ford-Walford plot (Gulland 1983) to facilitate comparison with earlier estimates of growth for this abalone. For the catch-curve analysis (Gulland 1983) we used the age-length data from commercial catch samples of 1994 and 1995 (N=221) to prepare an age-length key and applied that to length-frequency catch data from a midden believed to be of the late 1970s (N=228) on La Natividad and a sample (N=502) of March 1995.

Results

Microstructure

At low magnifications vertical sections of the shell of *H. corrugata* show clear growth rings near

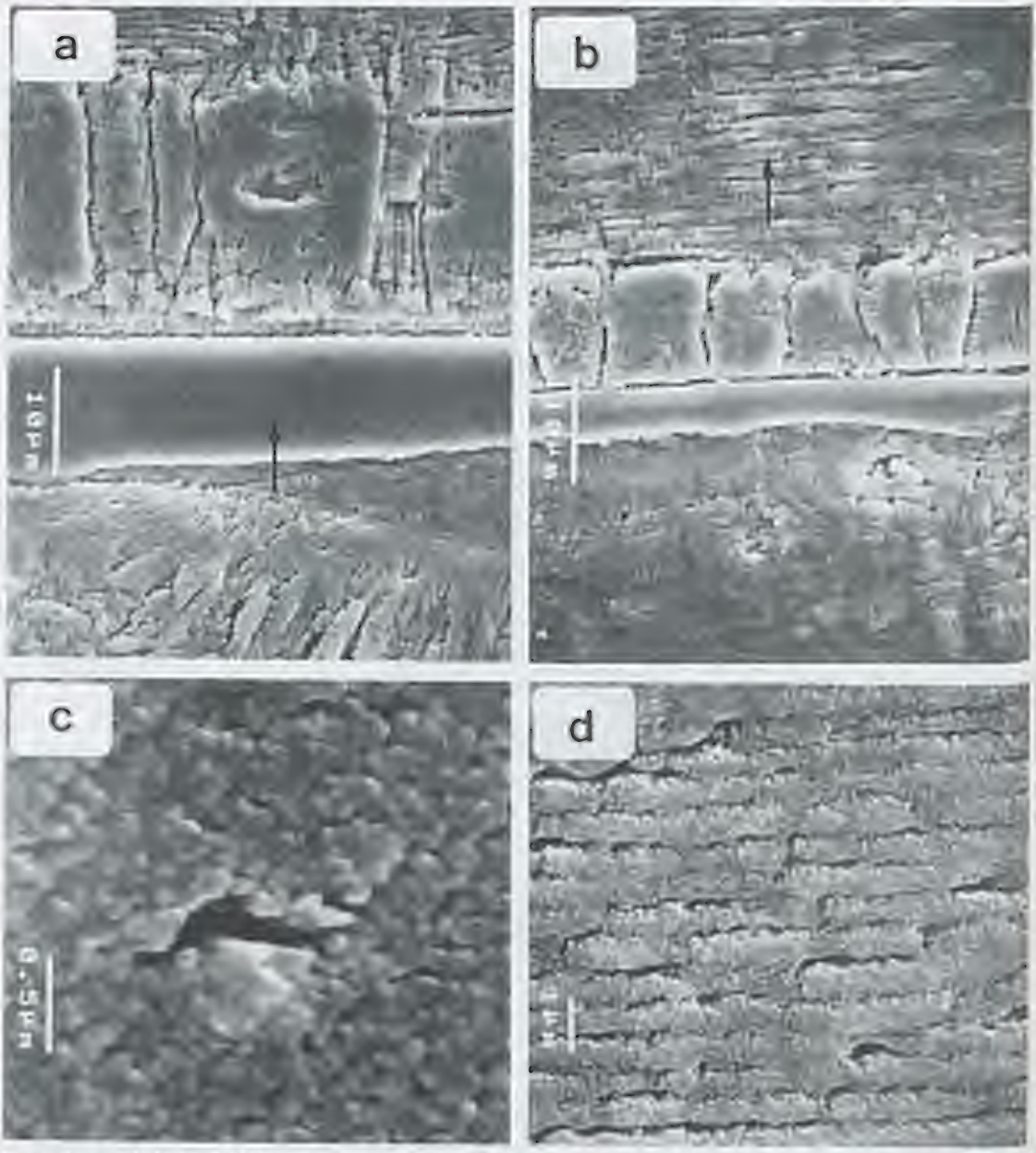

Figure 2. a – dark ring laid down at 8 years of age. The ring comprises block-like prisms above, and (arrow) layer of granular material below. b – dark ring laid down at 2–2 ½ years of age. Laminae of fairly uniform thickness (arrow) can be seen in the aragonitic nacreous layer laid down before the ring, whereas narrower, less regular laminae are laid down after the ring. Rings are laid down from top to bottom. c – micrograph of homogeneous granular material in lower part of ring. d – micrograph of laminae in aragonitic nacreous layer.

the spire (Fig. 1b,c). At higher magnification, up to three or four unpigmented rings (called fine rings) can be seen at the outer margin of the section close to the spire, but these disappear a short distance from it (Fig.1c). Pigmented rings (called dark rings) are then deposited sequentially toward the centre of the section and are conspicuous throughout the length of the section. SEMs show that

the rings have a structure not previously described for abalone. Initially, block-like prisms are deposited on the aragonitic substratum (Fig.2a,b) and then a dark layer of homogeneous granular material is deposited (Fig.2a,b,c) giving way again to aragonitic laminae. In contrast to *H. fulgens* and *H. rubra*, the laminae are fuzzy at the edges (Fig.2d) due to the coarseness of the marginal granules.

The electron micrograph sequence shows a striking cyclic ultrastructural pattern of variation in laminar thickness between rings (Fig.3). A plot of the change in mean thickness along a transect

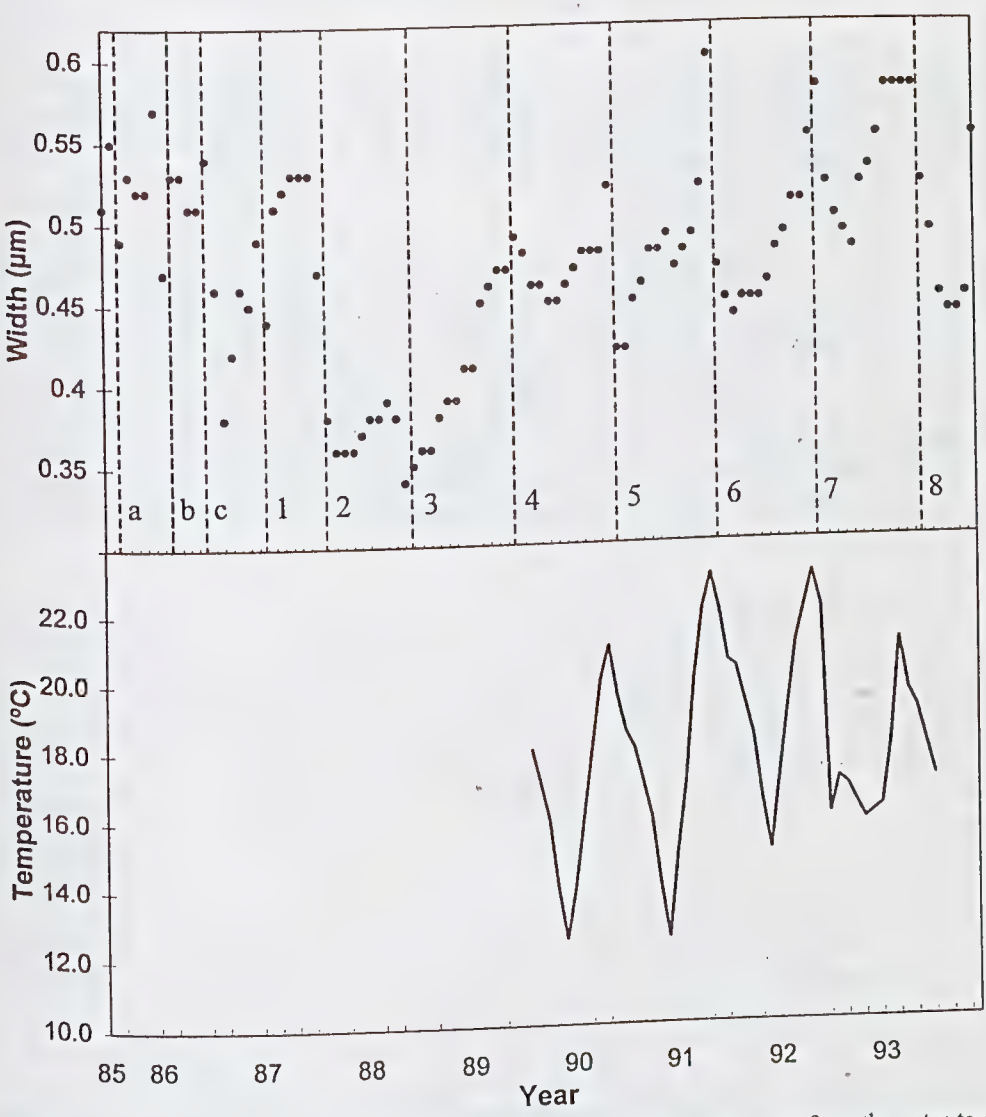

Figure 3. (upper) – plot of change in thickness of aragonitic laminae along a transect from the outer to inner shell surface in a vertical section at the spire of the shell of an 8 yr old animal captured in May 1994. Vertical lines show locations of rings, labelled with letters for fine ones, and with numbers for dark ones. The presumptive time of deposition is given on the x-axis on the assumptions that the dark rings after the first two are deposited annually in about October and that nacre deposition is uniform throughout the year. (lower) – sea surface temperatures for the Cedros- La Natividad region from January 1990 to December 1993 derived in part from Fernandez *et al* (1992) and in part from Gonzalez and Shepherd (1996).

Table 1. Parameters of regression equations of Gulland-Holt plots of annual increment (AI) vs mean length (L). The equations are of the form: AI = a – b L

Time after El Niño (years)	N	a (s.e)	b (s.e)	R^2
1	26	46.5(4.2)	0.28(0.02)	0.846
2.5	34	42.9(3.6)	0.28(0.02)	0.933
3.3	97	42.5(3.5)	0.24(0.02)	0.630

across the section is given in Figure 3. After the deposition of a dark ring the thickness of laminae declines abruptly and then increases again prior to deposition of the next ring. Lamina thickness was significantly correlated with sea temperature ($r=0.39$; $P<0.01$), and even reflected the higher temperatures during the El Niño of 1991–2 (Fig. 3).

Deposition of rings

We have no data on the timing of ring deposition other than in October and November. Nearly all shells taken during these months showed a new ring or part of one in the centre of the horizontal section, indicating very recent deposition. So we provisionally conclude that the dark rings are deposited in about October at about the time of maximum sea temperatures and also spawning.

A plot of the number of rings vs age of the shell for the shells grown in culture (Fig. 4) shows that three fine rings (a–c) are laid down in the first year, the first dark ring at about 20 months, the second soon after two years of age, and the third and fourth at three and four years respectively. A regression of the number of dark rings vs age gave a slope of 1.18 which did not differ significantly from unity ($t_{17}=1.96$; ns). The dark rings are thicker than the fine rings and are pigmented dark brown or black although paler shades also occur.

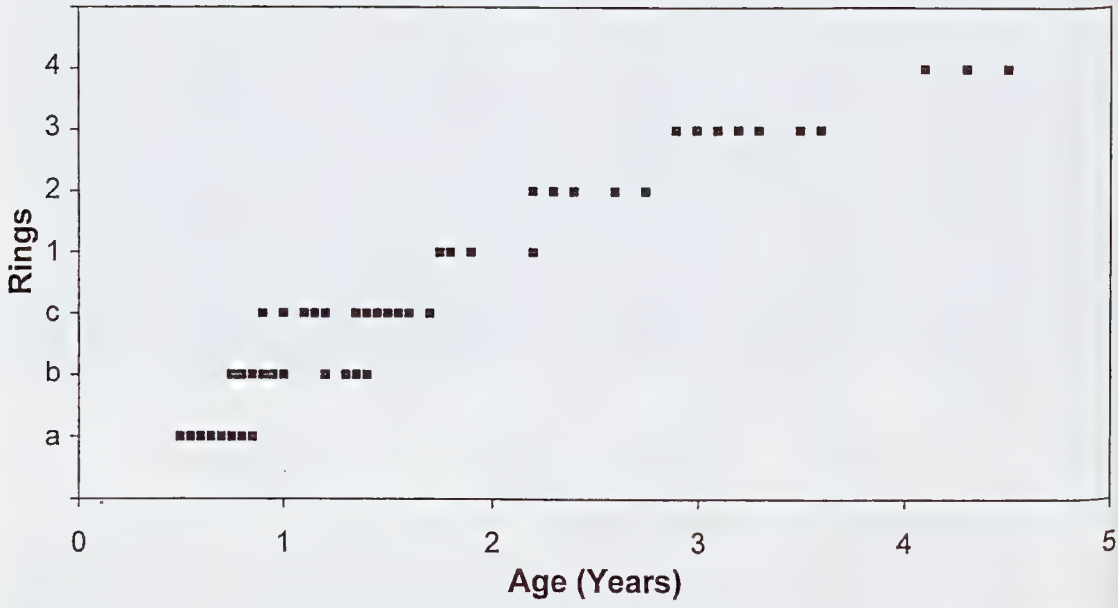

Figure 4. Plot of the number of rings vs estimated age of shells of *H. corrugata* reared in culture. a–c are fine rings and 1–4 are dark rings.

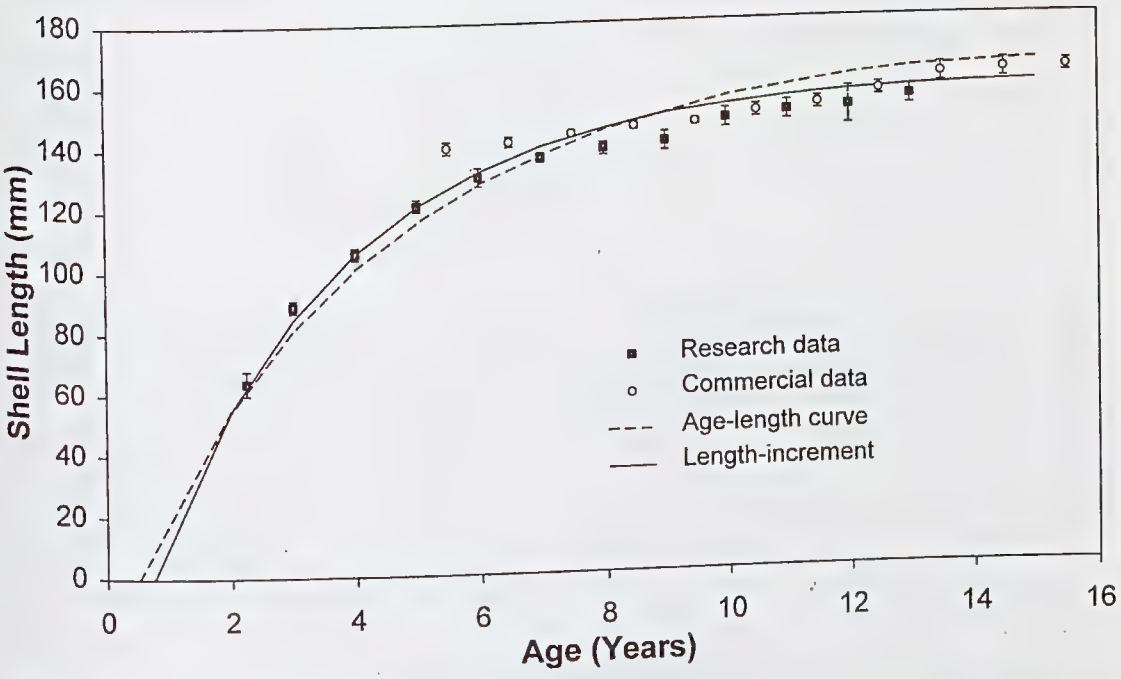

Figure 5. Plot of mean shell length vs putative age in years for data from research shell collections and from the commercial catch. Vertical bars are standard errors. The growth curve fitted to age-length data is shown with a continuous line, and that fitted to length-increment data is shown with a dashed line.

On the assumption that the same timing of deposition occurs in nature i.e. that from age 3 years the number of dark rings gives the number of whole years of age, we plotted mean shell length vs putative age (= number of dark rings) for (a) research shell collections, and (b) commercially caught shells (Fig. 5). Note that the mean length at age is initially higher for the commercial data than for the research data. The legal size at capture is 135 mm SL and the larger size of newly recruited shells presumably reflects the selection by divers of faster growing abalone. The parameters of the von Bertalanffy growth curve fitted to research and commercial data combined are given in Table 2, and plotted in Fig. 5.

Growth check analyses

Examination of 134 shells taken at La Natividad from 1989–1991 showed no prominent growth checks, whereas 50–65% of shells in samples taken from 1992–1995 showed a prominent growth check (Fig. 1a). However, shells with a presumed birth date in 1990 or later showed no growth check. So we concluded that the growth checks were laid down during the El Niño and hypothesised that they were deposited on 1 November 1991 as occurred in *H. fulgens* (Shepherd and Turrubiates 1997) soon after the time of maximum sea temperature. Hence the time periods from the date of deposition of the check would be approximately 1, 2.5 and 3.3 years for the shell samples taken in Nov. 1992, May 1994 and March 1995 respectively. We tested this hypothesis by comparing regressions of mean putative annual growth increment vs mean length (Gulland-Holt plots) for the three data sets. Neither the slopes ($t<1.9$; n.s.) nor the intercepts ($t<1.1$; n.s.) of the regressions differed significantly from each other (Table 1) so we accepted the hypothesis that the time periods were as postulated i.e. that the starting points, the time of deposition of the growth checks, were coincident on about 1 November 1991. Von Bertalanffy growth parameters calculated on the

Table 2. Parameters of von Bertalanffy growth equations for length-increment (LI) data and age-length (AL) data

Data	N	t_o	K yr^{-1} (s.e) (mm)	L_∞ (s.e.) (mm)	R^2
LI	157	0.46	0.254(0.014)	169.7(1.2)	0.73
AL	13	0.74	0.334(0.031)	159.6(14.0)	0.91

combined length-increment data are given in Table 2. Assuming that L=55 mm at age two, the growth curves of the two equations in Table 2 are presented in Figure 5 for comparison. Their trajectories are very similar and although they cannot be compared by formal statistics, their consistency supports the hypothesis that ring deposition is annual.

Next we plotted the observed number of rings vs mean estimated age in shells with growth checks (Fig. 6). Note that the standard errors of the mean ages increase with age after about 8 years. This is because estimates of age from length, while reasonable during the early nearly linear phase of growth, become increasingly noisy as the growth rate slows. The slope of the regression fitted to the mean estimated ages did not differ significantly from unity (t=1.6; n.s.); but if the means were weighted by the reciprocal of their standard errors in the regression, thereby reducing the weight of older ages, the slope of the regression is 1.11 which is significantly greater than unity (t=2.4; $P<0.05$). The results confirm that to age 12 one dark ring per year is laid down, with a possible accumulated bias of about one extra ring by about age 12 years.

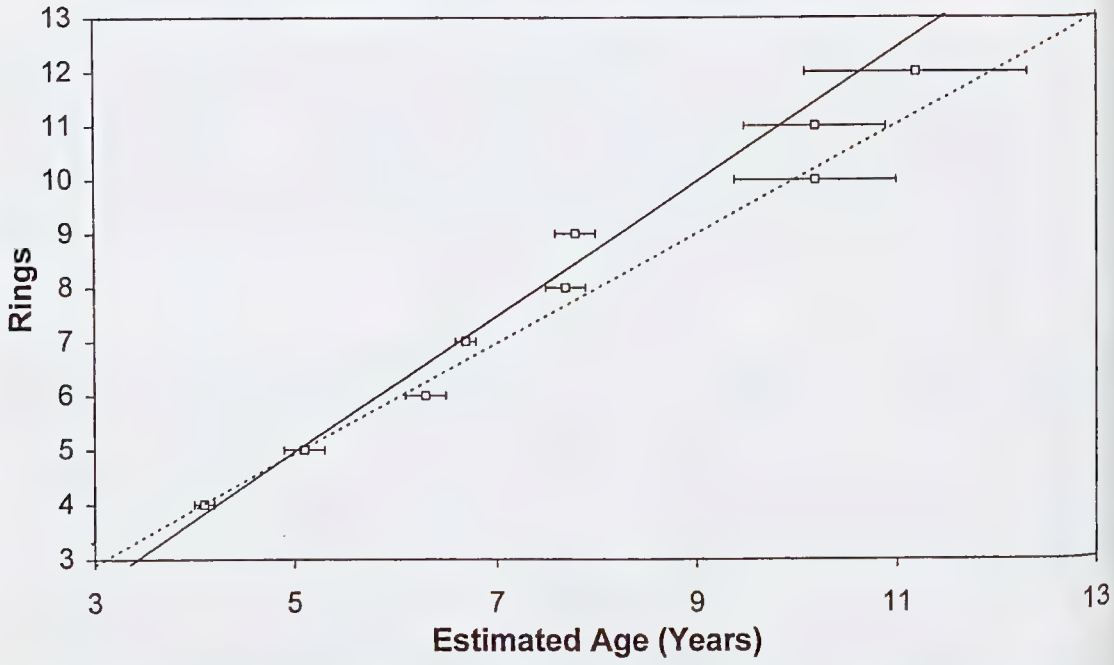

Figure 6. Plot of number of dark rings vs estimated age of shells with El Niño growth checks from the commercial catch taken in 1995. Horizontal bars are standard errors.

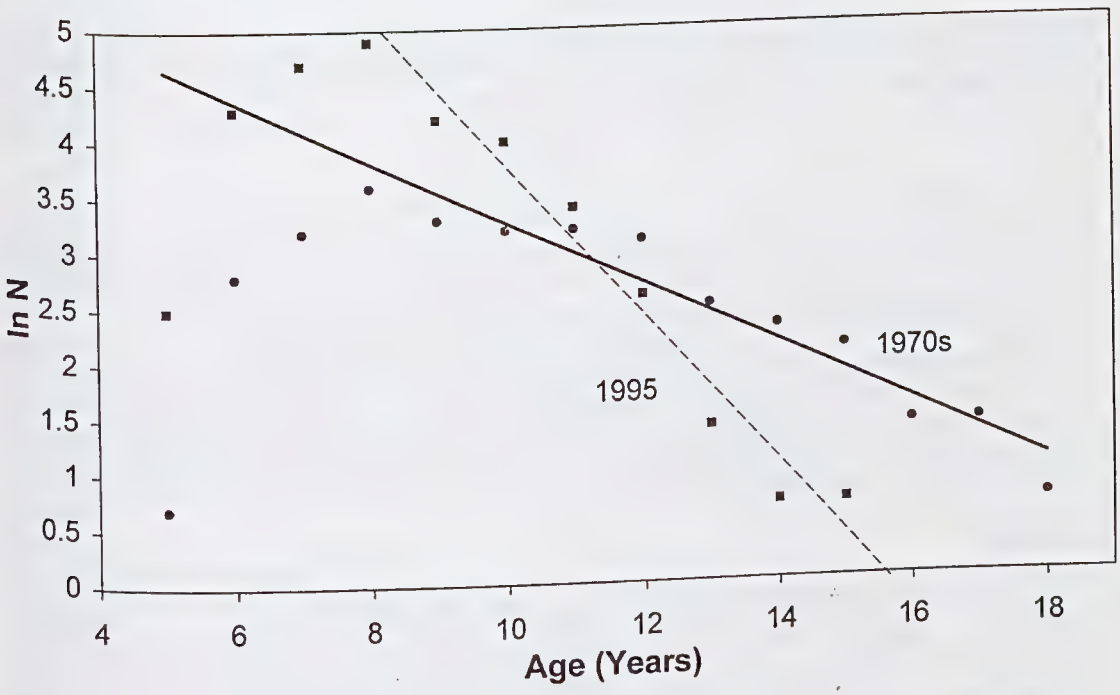

Figure 7. Catch- curve analyses for commercial catch data obtained in the late 1970s and in March 1995. Numbers in the 5–7 year age classes are not fully recruited to the fishery and are excluded from the regressions.

Total mortality rate (Z)

Plots of the natural logarithm of numbers in each age class vs age for the La Natividad samples (Fig. 7) gave estimates of Z of 0.28 (s.e. 0.02) for the late 1970s and 0.66 (s.e. 0.05) for 1995.

Discussion

Shell Microstructure

The microstructure of the rings and the pattern of changes in thickness of aragonitic laminae in *H. corrugata* differ from other species examined (Erasmus *et al.* 1994; Shepherd *et al.* 1995). The prismatic elements of the rings are much coarser and blocklike and the extra granular layer juxtaposed to the prisms has not been seen in other species. The latter is also strongly pigmented suggesting that it is rich in organic matter (conchiolin).

The cyclic change in thickness of laminae in *H. corrugata* is similar to that observed for *H. fulgens* (Shepherd *et al.* 1995a). In both species there is an abrupt decline in thickness after deposition of the dark ring which in both species appears to be laid down annually in October (see Shepherd and Turrubiates, 1997 for the most recent evidence on *H. fulgens*). In *H. corrugata* the correlation of laminar thickness with sea temperature suggests that the cyclic pattern is temperature-dependent as is known for bivalves (Lutz and Rhoads 1980). An alternative hypothesis is that the pattern is associated with maturation and spawning when the dark ring is laid down and laminar thickness declines abruptly. In support of this hypothesis note that the pattern in the first three years of life (following deposition of rings a–c, 1 and 3 in Fig. 3) before the age of sexual maturity differs

from that shown later. There are numerous published examples (reviewed by Day and Fleming 1992) where conspicuous interruptions to growth are associated with spawning or temperature extremes (and see also Shepherd *et al.* 1995b). Whatever the cause(s) of the cyclic pattern of changes in laminar thickness, they appear to provide internal evidence of the annual periodicity of ring deposition.

Growth checks

The occurrence of growth checks in abalone shells during strong El Niño events is considered by Shepherd and Turrubiates (1997). These checks are more pronounced in *H. corrugata* than in *H. fulgens*. Given the preference of *H. corrugata* for deeper and cooler water (Leighton 1974; Lindberg 1992; Guzmán del Próo 1992) we postulate that elevated temperatures during El Niños create relatively greater stress on this species than on *H. fulgens* and cause the more prominent check. However we cannot exclude a role of dietary change accompanying the disappearance of *Macrocystis* forests during strong El Niños. In this connection it is worth noting that the El Niño does not appear to have caused more than a brief interruption to growth since there was no significant difference in growth rates for the overlapping periods of 1, 2.5 and 3.3 years after that event (Table 1).

Ring counts

The rings in the spire of *H. corrugata* are very clear and give consistent counts. Horizontal sections are easier to prepare and read than vertical sections, although the latter show more clearly the occasional division of dark rings into two at the spire to produce a false ring (Shepherd *et al.* 1995a). However, with care false rings can be recognised in horizontal sections, too. The fine rings are readily distinguishable from dark rings because they are narrower and lack pigment. In any case they are progressively lost in older shells. But sometimes the first presumptive dark ring is unpigmented and could be mistaken for a fine ring especially in older shells. For this reason careful comparison of the two types of ring is important to avoid misclassification. If there are four (or more) initial fine rings present it is very likely that the excess over three are dark rings lacking pigmentation.

In other species of abalone extra "brown" rings may be deposited in response to parasitic attack (Shepherd and Huchette 1997, Shepherd and Triantafillos 1997)). In a few shells of *H. corrugata* we have seen "brown" rings which are wider and may be coloured more intensely than dark rings. Future studies need to address the question whether parasites can cause the deposition of extra rings in Mexican species as well.

Growth and mortality rates

The estimates of growth and mortality rates presented here are probably the most realistic values yet published for *H. corrugata* in Baja California. Earlier figures given for the region (reviewed by Guzmán del Próo 1992) ($K = 0.19–0.37$; $L_\infty = 159–198$ mm; $Z = 0.5–1.1$) have a wider range than ours, but cannot be critically evaluated because they are based on interpretation of modes of unpublished length-frequency data. Our low value for Z from the late 1970s is especially interesting in the light of the subsequent decline of this species in the 1980s. Thus, aging abalone shells provides the first opportunity in Mexico to obtain accurate parameter estimates for *H. corrugata* for use in age-structured models of the still valuable fishery.

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How many juvenile abalone are there? The example of *Haliotis roei*

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Key words: Abalone, *Haliotis*, juveniles, recruitment

Abstract

We use population surveys of *Haliotis roei* on intertidal rock platforms at Perth, Western Australia to examine recruitment of juvenile individuals into the adult, fishable population. Despite intensive surveys conducted seasonally and over several years very few small *H. roei* were found. We propose the following life cycle for *Haliotis roei*: juveniles grow fast, reach adult size quickly, and survive for a number of years as adults. Recruitment of juveniles to the adult population is low, either through a steady annual maturation of juveniles or by substantial recruitment during an occasional good year.

Introduction

A number of the larger species of abalone (genus *Haliotis*) combine to form one of the most commercially important groups of molluscs in the world. Abalone have attracted considerable research interest, and two major recent symposia were devoted entirely to the group (Shepherd *et al.* 1992; Shepherd *et al.* 1995).

Very high levels of exploitation have caused the collapse of a number of major abalone fisheries, including those in California (Tegner *et al.* 1992; Parker *et al.* 1992) and Mexico (Guzman del Proo 1992). Understanding the reasons for the collapse of abalone populations supporting wild fisheries is essential for the sustainable management of the remaining commercially fished populations. In this paper we argue that recruitment of juvenile abalone into a population may be substantially lower than currently thought, thus increasing the chance of recruitment failure.

Size structure in surveyed populations of some abalone species is typically skewed to the right with a preponderance of accumulated year classes and very few juveniles (Tegner *et al.* 1992; Wells and Keesing 1989; 1990; Wells and Mulvay 1995). It is frequently assumed that the lack of juveniles found in surveys is a sampling artefact caused by the inability of researchers to effectively sample juveniles in cryptic habitats. There is an alternative possibility: that juveniles are not abundant but grow fast, quickly reach adult size, and survive for many years as adults. Recruitment of juveniles to

the adult population may thus be low, and heavily dependent on levels of natural mortality, known to be high. If this is true, fished populations will be largely based on occasional years when recruitment is high. Fisheries with these characteristics are susceptible to growth overfishing.

The present paper examines available information for *Haliotis roei* Gray to examine whether the rate of recruitment of juveniles to the fishable population is characterised by low levels of steady recruitment or high interannual variability. Juveniles of *H. roei* are relatively easily sampled on intertidal limestone platforms in the Perth area as there are few places for them to hide and the growth characteristics are well known (Keesing and Wells 1989).

Materials and methods

In the Perth metropolitan area of Western Australia *H. roei* is abundant near the seaward margin of intertidal limestone platforms. The platforms studied are flat at about low tide level and extend seaward for up to 100 m from the shoreline. The platform margin may be raised a few centimetres, trapping water on the platform during low tide. At the seaward margin the platform drops vertically before reaching the bottom of sand or rock at a depth of several metres; the vertical dropoff may be undercut. Few, if any loose rocks occur on the platforms. The surface is irregular, with holes several centimetres across and deep on the entire surface. In some areas near the platform margin solution pipes may penetrate through the platform to the undersurfaces. Crustose coralline algae occur only near the seaward margin of the platform. Grazing molluscs, particularly limpets, keep many parts of the platform surface near the margin largely free of macroalgae (Scheibling *et al.* 1990). The subtidal vertical cliff at the seaward margin of the platform is relatively smooth. Thus there are few hiding places for small abalone.

Data collected from two platforms are examined here. Waterman has been a fishery reserve since the late 1960s and has not been subjected to fishing. In contrast, the platform 3 km to the south at Trigg has been extensively fished over many years, originally by professional then later by recreational fishers. The platforms have a clear habitat zonation (see Wells and Keesing, 1986): the inshore platform has a variety of macroalgae in summer which are removed by winter wave action; the *Sargassum* zone with large macroalgae which persist during winter; and a bare zone near the seaward margin of the platform where few macroalgae are present.

Detailed sampling of all molluscs present was undertaken in January of each year from 1983 to 1986. In each habitat a transect of 8 stations 5–10 m apart was sampled. At each station all molluscs 5 mm or more in shell length (SL) counted and as far as possible identified in the field. Abalone spawn in the winter months of July and August, and reach a shell length of 46 mm after one year. Individuals < 20 mm SL sampled in January would thus be 0+ animals spawned during the previous winter.

Two surveys were undertaken to measure the small scale distribution of abalone. To examine horizontal distribution of abalone on the platform, 6 transects were made perpendicular to the seaward edge of the platform at Trigg. The transects were 10 m long and stretched from the seaward edge of the platform into the *Sargassum* zone. Every metre a 50 x 50 cm quadrat was examined and all abalone counted and measured in situ with callipers. A second quadrat, adjacent to the first, was subdivided into a grid and used to estimate the cover of crustose coralline algae.

A site on the cliff face at the seaward edge of the platform at Waterman was used to measure vertical distribution of *H. roei* on the seaward subtidal cliff. Five transects were run from the top of the platform, at about the low tide line, to the bottom at a depth of about 1.8 m. Quadrats were 50 cm wide and 30 cm high.

Size frequency measurements were made quarterly from January 1983 to January 1985 at 2 sites in the bare zone at Waterman and 3 sites at Trigg. A 50 x 50 cm quadrat was placed in the bare zone and all abalone measured to the nearest millimetre with callipers. A total of 20 quadrats were haphazardly sampled at each site. Because of the exposed position of the sites, some quarters were missed at some sites during the winter.

To examine repopulation of abalone from a denuded reef, all abalone were removed from a subtidal rock approximately 2 m in diameter and extending 1.8 m from the sand bottom to the surface in the Waterman reserve in March 1983. All abalone visible were removed daily for a period of 5 days. The number of abalone collected declined rapidly after the first day, with fewer than 20 animals collected on the last day. Visual observations were also made in November 1983 and March 1984. The rock was cleared again in March 1985 and 1987.

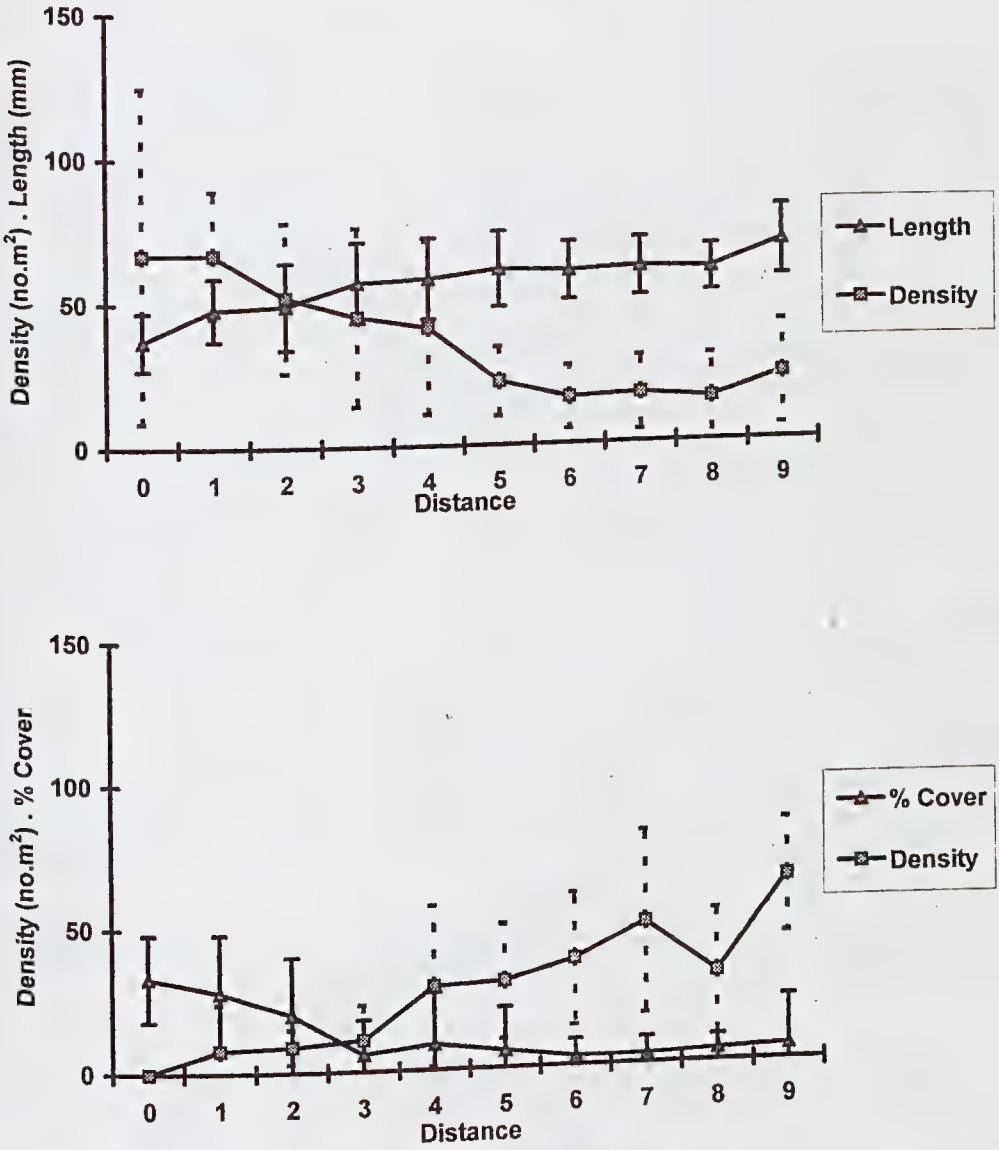

Figure 1. Horizontal changes in mean shell length and density of the abalone *Haliotis roei* (upper) and the density of *Sargassum* and the percentage cover of coralline algae (lower) with increasing distance from the seaward edge of the platform at Triggs. Means 1 S.E. are shown.

Results

Abalone are concentrated at the seaward margin of the platform. During the four years of intensive sampling on the inshore platform a total of 1961 molluscs were identified and counted at Waterman and 117,477 at Trigg, but not a single specimen of *H. roei* was encountered on the inshore platform at either Waterman or Trigg (Table 1). Some abalone were found in the *Sargassum* zone, primarily the portion closest to the adjoining bare zone. However, only 213 abalone (0.3%) were present of the total of 69,848 molluscs counted in the *Sargassum* zone at Trigg. Both the total number of abalone (579) and the percentage (6.7% of 8602 molluscs) were substantially higher in the *Sargassum* zone at Waterman. This may be a result of individuals forced by the high abalone density in the bare zone to move into adjacent suboptimal habitats. The greatest number of abalone at both Waterman (3763) and Trigg (819) were in the bare zone at the seaward margin of the platform, where they comprised the greatest percentage of total molluscs (21.4 and 4.2% respectively).

Figure 1 shows the density of *H. roei* decreased rapidly with increasing distance from the seaward edge of the platform at Trigg. Maximum densities of 66 m^{-2} occurred at the seaward margin and declined to a consistent level of 17 to 24 m^{-2} on the inshore stations. Mean size of *H. roei* was lowest (36 mm) at the seaward margin and increased gradually to 69 mm at 9 m from the seaward edge.

Linear regressions of abalone density and mean size against percent cover of coralline algae and density of macroalgae showed that density of *H. roei* was correlated with percentage cover of coralline algae ($r= 0.52$, $p<0.001$) and conversely with macroalgal density ($r= -0.39$, $p<0.01$). Mean size of abalone was also conversely correlated with percentage cover of coralline algae ($r= -0.41$, $p<0.01$). The relationship between mean abalone size and macroalgal density was not significant.

The population density of *H. roei* also changes vertically (Figure 2). Density was greatest (108 m^{-2}) at the top of the cliff and decreased sharply with increasing depth. Mean size increased with increasing depth from 57 mm at the top to 87 mm at the 90–120 cm level. Visual observations in other areas confirm that this is a general pattern, with H. roei being most abundant in wave-swept areas near the ocean surface.

A total of 762 *H. roei* were collected from an isolated rock in the Waterman reserve in March 1983 (Figure 3; Table 2). The size frequency histogram was strongly skewed towards larger individuals;

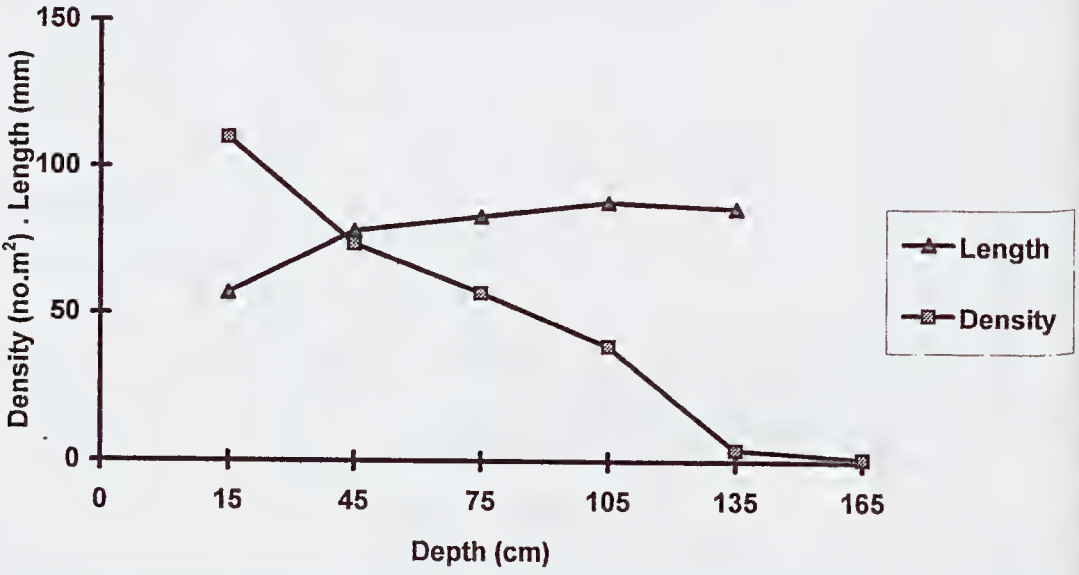

Figure 2. Vertical changes in density and mean size of the abalone *Haliotis roei* with increasing depth on the seaward vertical cliff at Waterman. Means 1 S.E. are shown.

Table 1. Total numbers and density of abalone (*Haliotis roei*) in censuses conducted on intertidal rock platforms at Waterman and Triggs annually in January 1983 to 1986.

	Waterman							
	1983		1984		1985		1986	
	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)
Inshore platform	0	0.0	0	0.0	0	0.0	0	0.0
Sargassum zone	387	48.4	149	18.6	12	1.5	31	3.9
Bare zone	1000	125.0	830	103.7	917	114.6	1016	127.0
	Trigg							
	1983		1984		1985		1986	
	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)	No.	Density ($no.m^{-2}$)
Inshore platform	0	0.0	0	0.0	0	0.0	0	0.0
Sargassum zone	77	9.6	41	5.1	34	4.3	61	7.6
Bare zone	123	15.4	178	22.3	249	31.1	269	33.6

537 animals, or 70.5% were in the 2+ age group, and most were probably several years old. Only 17.0% of the population was in the 0+ age class and 12.5% in the 1+ age class. Collecting was done every morning for five days to ensure small individuals were sampled. As the large individuals were removed, vacated spaces were occupied on subsequent days by small individuals which were then easily collected. Because of the intensive collection by two divers over a number of days, we believe the low representation of 0+ and 1+ individuals was real and not a collecting artefact.

The rock was visited at irregular intervals for the next two years. During late summer, when the clearing was undertaken, the rock was separated from an adjoining larger rock by sand. Sand between the rocks was scoured out by wave action during winter and a bare limestone base exposed, allowing mature abalone to migrate from the high densities of the unsampled rock to the cleared sample rock. Thus when the sample rock was cleared again in 1985 and 1987 adult abalone were present. Young abalone could have migrated in either direction. Since they are much less dense and are less mobile than adults the migration is thought to have been slight. In March 1985 a total of 294 *H. roei* were removed from the rock; only 141 were less than 2 years old. In March 1987 151 animals were in this age category. Thus, 0+ and 1+ age classes were sampled for a total of 6 years and a total of 516 animals collected. The 0+ and 1+ year classes averaged only 11.2% of the total original population. At this rate the population would replace itself in 9 years if natural mortality of 2+ animals was zero.

Table 2. Age and size structure of *Haliotis roei* collected on a small rock at Waterman. Determination of ages is based on growth rates provided by Keesing and Wells (1989). Percentages are of the original (1983) total density.

Age (years)	Size (mm)	1983		1985		1987	
		No.	%	No.	%	No.	%
0+	<45	129	17.0	90	11.8	83	10.9
1+	45–65	95	12.5	51	6.7	68	8.9
2+	>65	537	70.5	153	20.1	142	18.5
Totals		761	100.0	294	38.6	293	38.5

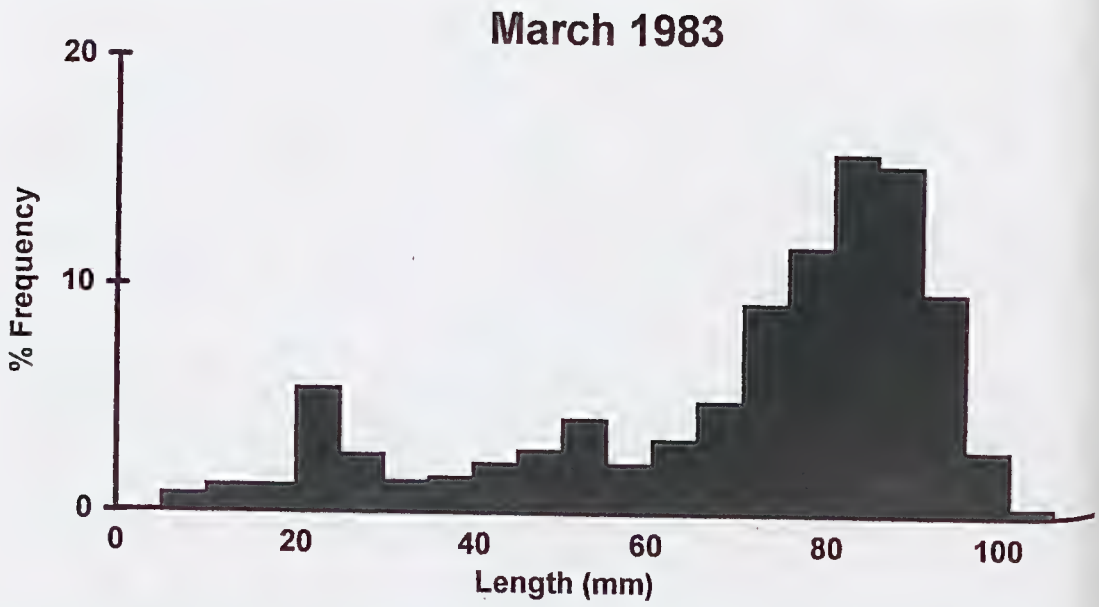

Figure 3. Size frequency characteristics of *Haliotis roei* collected on an isolated rock at Waterman in March 1983.

The three sites, two at Trigg and one at Waterman, with the most complete records for the quarterly size frequency measurements are shown on Figure 4; results obtained at the two additional sites were similar. The *H. roei* population at Waterman South was dense (usually between 90 and 100 m^{-2}) and largely adult. The minimum recorded was 62.4 m^{-2} in April 1984 and the maximum was 184.2 m^{-2} in January 1985. Densities of animals <20 mm were always less than 8.0 m^{-2}. Total abalone density was substantially lower at both sites at Trigg, as a result of fishing pressure. At Trigg North densities of adults were between 30.6 and 53.0 m^{-2}. Densities of animals <20 mm ranged from 2.9 to 9.5 m^{-2}, except for January 1985 when they were 19.0 m^{-2}. At Trigg West the range was 35.0 to 88.0, with animals <20 mm <10 m^{-2} except for densities of 18.0 m^{-2} in January 1984 and 14.7 m^{-2} in July 1984.

The sites were also visited annually in January from 1983 to 1986 (Figure 5). In all cases densities of animals <20 mm were very low compared to total densities. Total densities at Waterman varied from 95.8 to 184.2 m^{-2}, but animals <20 mm never exceeded 5.6 m^{-2}. Densities were much lower at Trigg, from 21.4 to 61.0 m^{-2}, with densities of animals <20 mm being up to 19.0 m^{-2}.

Adult animals were collected from the subtidal cliff at the seaward margin of the platform from January 1983 to January 1985 and 40 individuals were recovered each from Trigg and Waterman. During this collecting the subtidal cliff was surveyed for small abalone, but no large concentrations were found.

Discussion

There has been intensive study of molluscs on intertidal rock platforms and the fishery biology of *H. roei* in the Perth metropolitan area (Wells and Keesing 1986; 1989; 1990; Wells *et al.* 1989; Keesing and Wells 1989). In the area studied *H. roei* has a very high growth rate, reaching 46 mm SL in the first year, 64 mm SL in the second year, when they are sexually mature, and 71 mm SL in the third. The total lifespan is not known. Growth rates are substantially higher on the subtidal cliff than on the nearby intertidal platform (Keesing and Wells 1989).

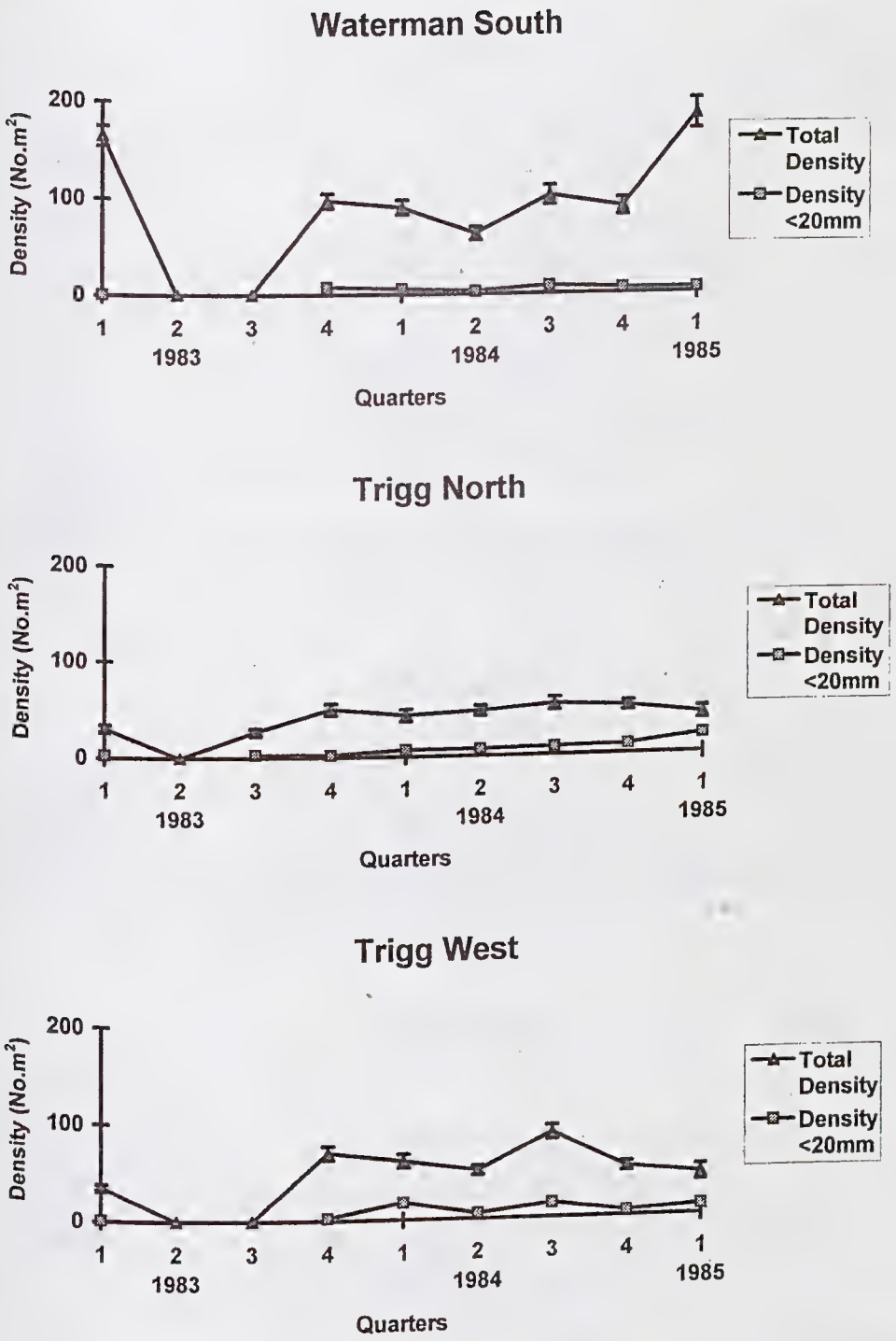

Figure 4. Comparison of total density and density of *Haliotis roei* smaller than 20 mm at three sites on intertidal platforms at Trigg and Waterman at quarterly intervals from January 1983 to January 1985. Means 1 S.E. are shown.

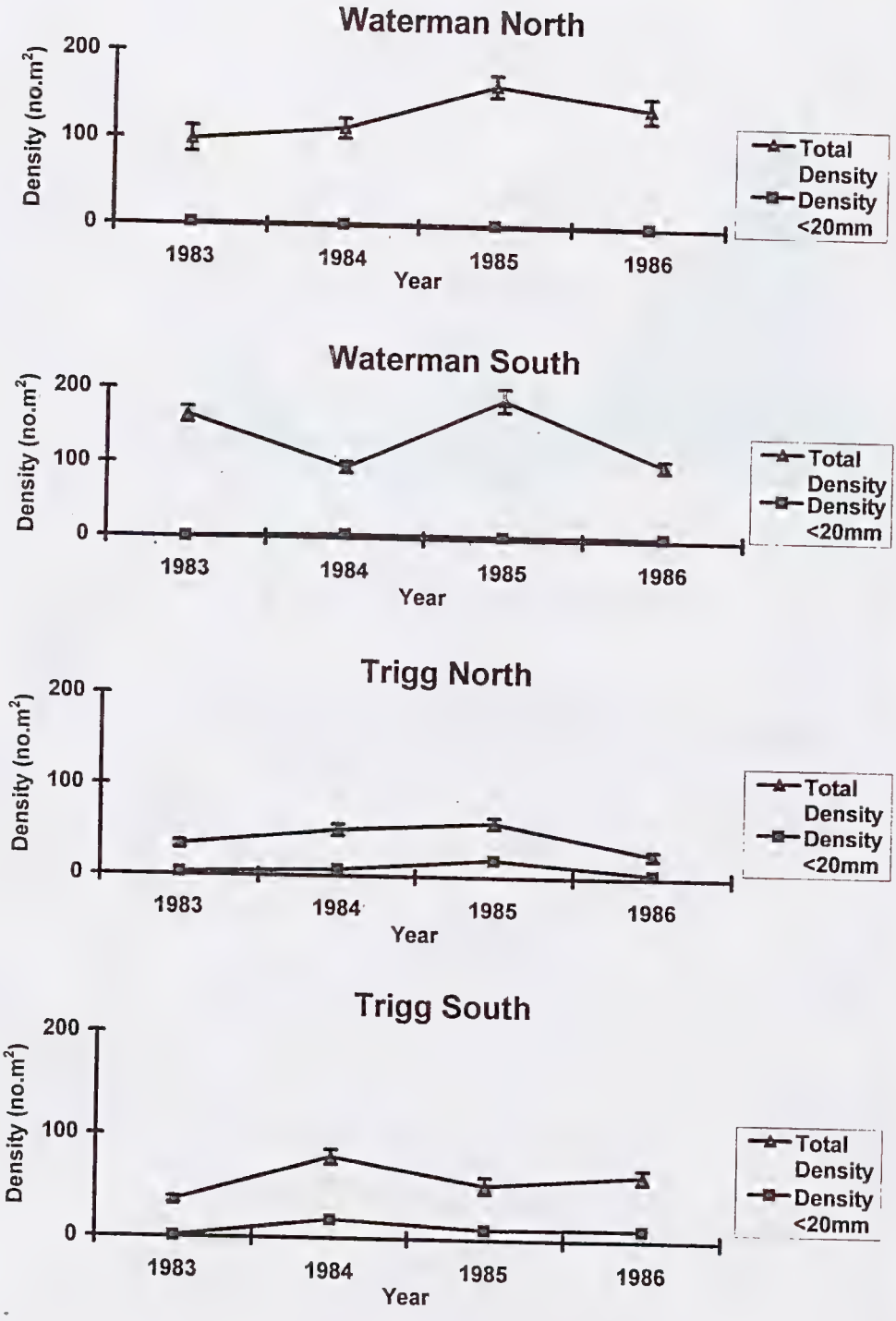

Figure 5. Comparison of total density and density of *Haliotis roei* smaller than 20 mm at four sites on intertidal platforms at Trigg and Waterman at annual intervals from January 1983 to January 1986. Means 1 S.E. are shown.

When we began sampling in the Waterman reserve in 1983 the population of *Haliotis roei* was maximal with virtually all available sites occupied by adult abalone. Young individuals appeared to be more mobile, suggesting they were occupying suboptimal positions and were moving about regularly in search of a better site. Population density was thus apparently stable. We believed young individuals could recruit only when space became available. In comparison, many vacant spaces were present on the rock platform at Trigg, which was under considerable fishing pressure. Despite the vacant habitats, quarterly and annual data show little difference in the levels of small individuals on the two platforms.

We propose the following life history strategy for *Haliotis roei*: juveniles grow fast, reach adult size quickly, and survive for a number of years as adults. Recruitment of juveniles to the adult population is low, either through a steady annual maturation of juveniles or by substantial recruitment during an occasional good year which sustains the adult population through a number of years when recruitment is very low. If this scenario is true, the lack of juveniles in the population will make *H. roei* particularly susceptible to growth overfishing and potentially recruitment overfishing.

Although recruitment for *H. roei* has been shown to be low, the rapid growth rate allows juveniles to become adults within two years. The relatively short juvenile phase reduces juvenile mortality, and allows the maintenance of substantial adult populations as shown during the rock clearing experiment.

McShane (1995) has examined in detail the literature on recruitment various species of abalone and concluded differences in recruitment can be large. Recruitment is low in species such as *H. laevigata* and *H. iris*, but is high in *H. rubra* and *H. discus hannai.* It is interesting that Shepherd (1990) demonstrated recruitment failure in an unfished population of *H. laevigata.* These studies indicate the substantial need for further data on the life history strategies of other species of abalone.

Acknowledgments

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A practical chronology for the abalone *Haliotis fulgens*

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"The smallest spiral holds the history
Of something tiny in the battering sea"
The Seashell, Judith Wright

Abstract

The abalone *Haliotis fulgens* deposits fine (non-pigmented) and dark (pigmented) rings in the spire that are useful for aging the shell. A dark ring is deposited annually, and in the first 4–5 years is preceded by one or more fine rings which are deposited with decreasing frequency over time. External shell erosion causes the loss of about one ring per 32 mm shell length over about 80 mm length. The El Niño events of 1982–3 and 1991–2 caused growth checks in shells of *H. fulgens* at La Natividad and Bahia Tortugas, Baja California and were used as a time-stamp to validate the rate of deposition of dark rings in the shell. In about 9% of the shells examined more or less dark rings are deposited than expected. In these cases counts of fine rings as well as dark rings can reduce the chance of error.

Keywords: Haliotis fulgens; rings; chronology; shell-aging; El Niño.

Introduction

The ability to age abalone shells is a valuable tool for management of their fisheries. Validation of the technique has been problematic but has advanced with ultrastructural studies (Erasmus *et al.* 1994; Shepherd *et al.* 1995a) and the use of time-stamps such as natural growth-checks or artificial fluorochromes (Shepherd *et al.* 1995b; Day *et al.*1995).

After the discovery of rings in the spire of abalone shells by Hayashi (1955), Muñoz-Lopez (1976) proposed their use in aging shells of *Haliotis fulgens* Philippi which deposits non-pigmented rings (called fine rings) and pigmented rings (called dark rings). However he neither specified the type of ring he counted nor did he validate the method. Shepherd *et al.* (1995a) showed that *H. fulgens* deposits on average 4 rings in the first year and 3 rings in each of the next 3 years but they likewise did not distinguish the two types of ring and they only examined the rate of deposition of rings during the first 4–5 years of age. Turrubiates and Castro (1992) counted only the dark rings to age the shell, assuming a rate of one a year, and found fair agreement between growth rates estimated from mark-recapture studies and those from shell-aging. In this paper we evaluate Turrubiates and Castro's (1992) method. We re-examined the same shells aged by Turrubiates and Castro, as well as other samples, and describe the pattern of deposition of fine and dark rings.

The El Niño oceanographic events of 1982–3 and 1991–2 were both strong episodes which

elevated sea temperatures by 2–3°C in Baja California and eliminated for a year the giant kelp *Macrocystis pyrifera* from the region. These events are known to cause prominent growth checks in abalone shells (Cox 1962) and they did so in abalone shells in this region (Shepherd and Avalos-Borja 1997). We estimated the date of deposition of "EL Niño" growth-checks in 1983 and 1991 in *H. fulgens* and used them as time-stamps to determine the rate of deposition of dark rings, and so confirm the validity of Turrubiates and Castro's aging technique.

Methods and materials

We examined a sample of 75 shells taken from Bahia Tortugas (27°40' N; 114°52' W) in October 1984, first studied by Turrubiates and Castro (1992), and a second sample of 72 shells taken commercially from La Natividad (27°51'26" N; 115°7'30" W) in March 1995. The spires of the shells were ground down with an electric grinder and polished with 200–600 grit abrasives until a minute hole appeared (termed a horizontal section) as described by Shepherd *et al.* (1995a). The rings exposed by this section were counted from the outer margin to the central hole, and recorded as: f_1, d_1; f_2,d_2;....f_n,d_n where f_n is the number of fine rings immediately preceding the nth dark ring d_n. Shells showing aberrant patterns e.g. long (6–8) initial sequences of fine or dark rings were excluded from the analysis.

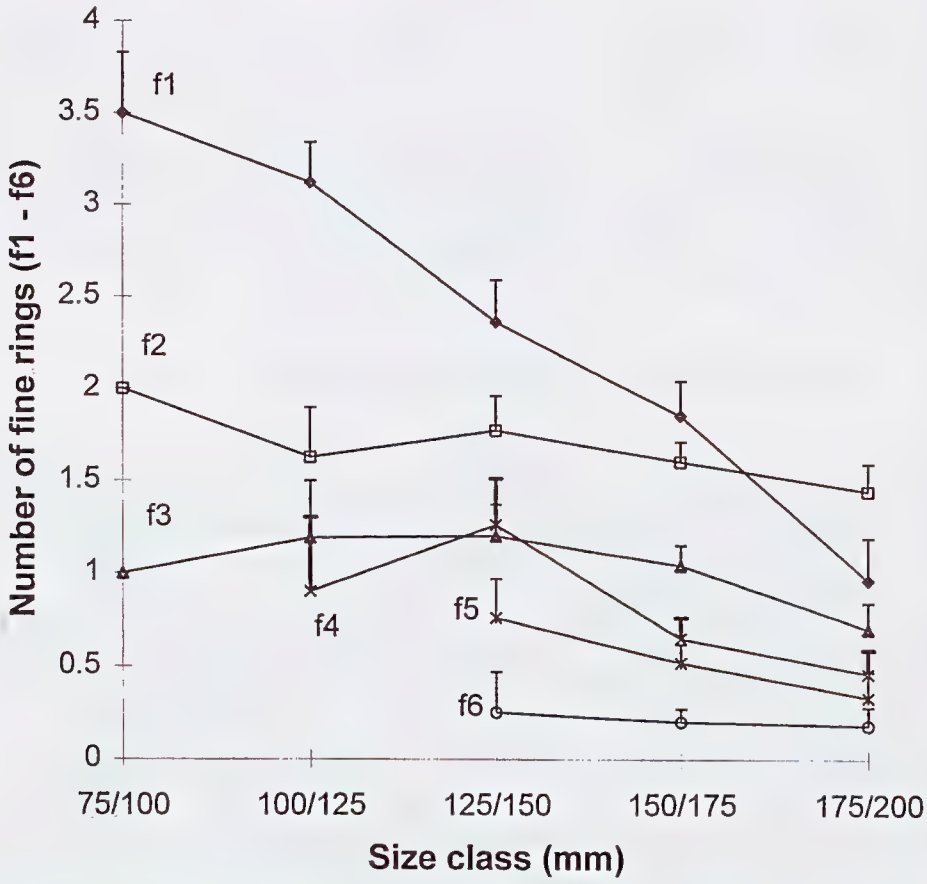

Figure 1. Plot of mean number of fine rings, f_1-f_6, preceding the dark rings, d_1 to d_6 for five different size classes of shell length. Vertical bars are standard errors. Sample sizes are: f_1, n=143; f_2, n=146; f_3, n=150; f_4, n=133; f_5, n=111; f_6, n=72.

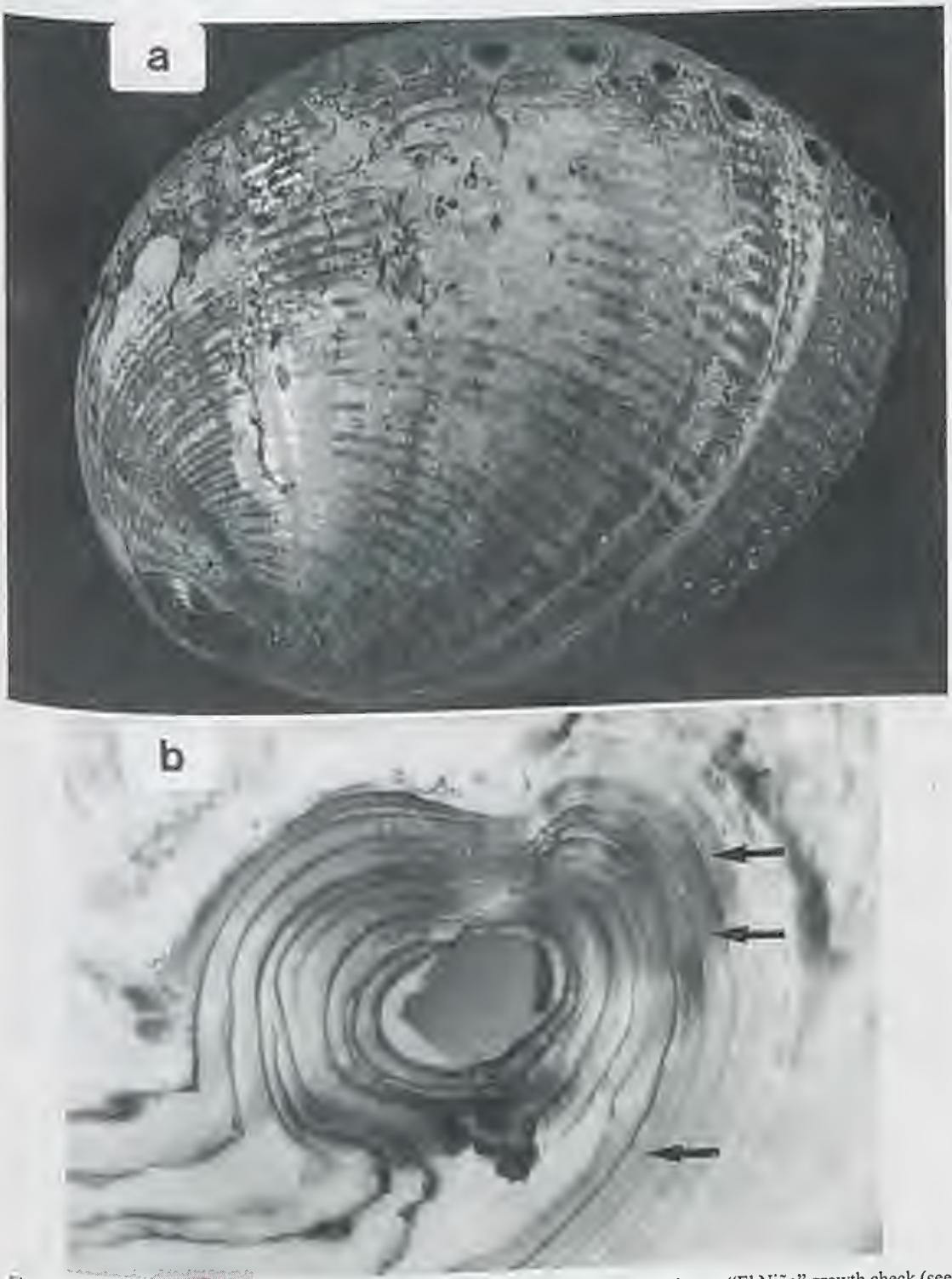

Figure 2. **a**. Photograph of shell (159 mm SL) of *H. fulgens* from La Natividad showing an "El Niño" growth check (see arrowhead) at SL_t = 137 mm. **b**. Photograph of horizontal section of the same shell showing sequence of dark rings with 1–3 fine rings (indicated by arrows) between the outer dark rings.

From the above samples we selected shells showing a prominent growth check and measured shell length to the growth check (SL_1) and total shell length (SL) in mm and counted the number of dark rings. To estimate the date of deposition of the growth check we selected a subset of the shells where 100mm<SL<148 mm. Although the aging technique of Shepherd *et al.* (1995a) has been strictly validated only to 130 mm SL the data suggested validation could be safely extrapolated for another year to 148 mm SL. That technique counts fine and dark rings and then applies an equation relating age and number of rings after taking into account loss of rings through shell erosion. We assumed a uniform birthdate on 1 November in accordance with the known time of spawning (Ortiz *et al.* 1990), and estimated the year of birth of each shell. Assuming a linear growth rate of 32 mm per year to 128 mm SL and 20 mm in the 5th year (Shepherd *et al.* 1995a) we obtained, by interpolation, an estimate of the age (A_1) when the growth check (SL_1) was laid down, and hence the date of deposition. We then estimated the age of shells >148 mm SL with "El Niño" growth checks by a two step procedure i.e. by first estimating the age to SL_1 as described, and then by adding the time period from the mean date of deposition of the growth check to the date of capture of the shell. For each site the number of dark rings was plotted against the estimated age of each shell, and the data analysed by least squares regression.

Growth rates were examined by two independent methods at each site. First, the El Niño growth check data were used to estimate von Bertalanffy growth parameters by plotting the increment ($SL-SL_1$) vs mean length ($SL_1+SL)/2$) which is the Gulland- Holt plot (Gulland 1983). Second, the age-length data obtained from aged shells were analysed by Ford- Walford plots (Gulland 1983). These simple analyses were used for comparison with published growth parameters derived by the same methods at the same sites. The length-increment data for Bahia Tortugas also included data from three tagged shells recaptured 1–3 years after marking.

Results

Rings

A plot of the mean number of fine rings preceding the first 6 dark rings vs shell length (grouped for ease of presentation in 4 size classes) (Fig.1) shows that a mean of 3.5 rings (range 2–6) are initially laid down before the dark ring d_1, about two before d_2, one before d_3 and d_4, and thereafter fine rings are seldom deposited. A regression of f_1 (the number of fine rings preceding the first dark ring) vs shell length gave the equation: $f_1 = 5.0 - 0.020$ SL ($r^2 = 0.42$) (n=118) indicating an average loss of one ring per 32 mm over the length range 75–200 mm SL. None of the slopes of the regressions of f_2, f_3,....f_6 vs shell length were significant (in each case t< 1.5 n.s.). In 7% of the shells we observed a sequence of up to 8 fine rings before a dark ring indicating that no dark rings were laid down in the first 2 years. Conversely in 2% of the shells we observed a sequence of dark but no fine rings indicating that in the first 2–3 years scarcely any fine rings were laid down. A typical shell with sequences of fine and dark rings is shown in Figure 2b. Many of the shells taken in October or November samples showed partial or very recent deposition of a dark ring suggesting that the dark ring is deposited around spawning time.

Table 1. Parameters of regression equations of observed number of rings (R) vs predicted age (A) in years. The equation is of the form R = a + b A

	a	b	s.e	r^2	N
La Natividad	–0.33	1.05	0.06	0.90	47
Bahia Tortugas	–0.39	1.08	0.05	0.98	22

Growth checks

An example of an "El Niño" growth check is shown in Figure 2a. For the 1995 sample from La Natividad 16 shells 135–148 mm SL showed a prominent growth check. The mean date of deposition of the check was on 18 November 1991 (s.e.10 days). For the sample from Bahia Tortugas there were 11 such shells 102–148 mm SL. The mean date of deposition of the growth check was on 4 October 1983 (s.e. 9 days). Samples of shells (N=47) from La Natividad taken from 1989–1991 showed no such growth checks. Plots of the observed number of dark rings vs the estimated age are given in Figure 3 and the parameters of the fitted regression equations are given in Table 1. The mean period "at liberty" i.e. from the date of the growth check to the date of capture was 3.3 years at La Natividad; for the Bahia Tortugas sample the period was one year for 15 shells captured in October 1984 and 7 years for 5 shells captured in November 1990. In both cases the 95% confidence interval of the regression slopes include unity indicating that on average one dark ring is deposited annually.

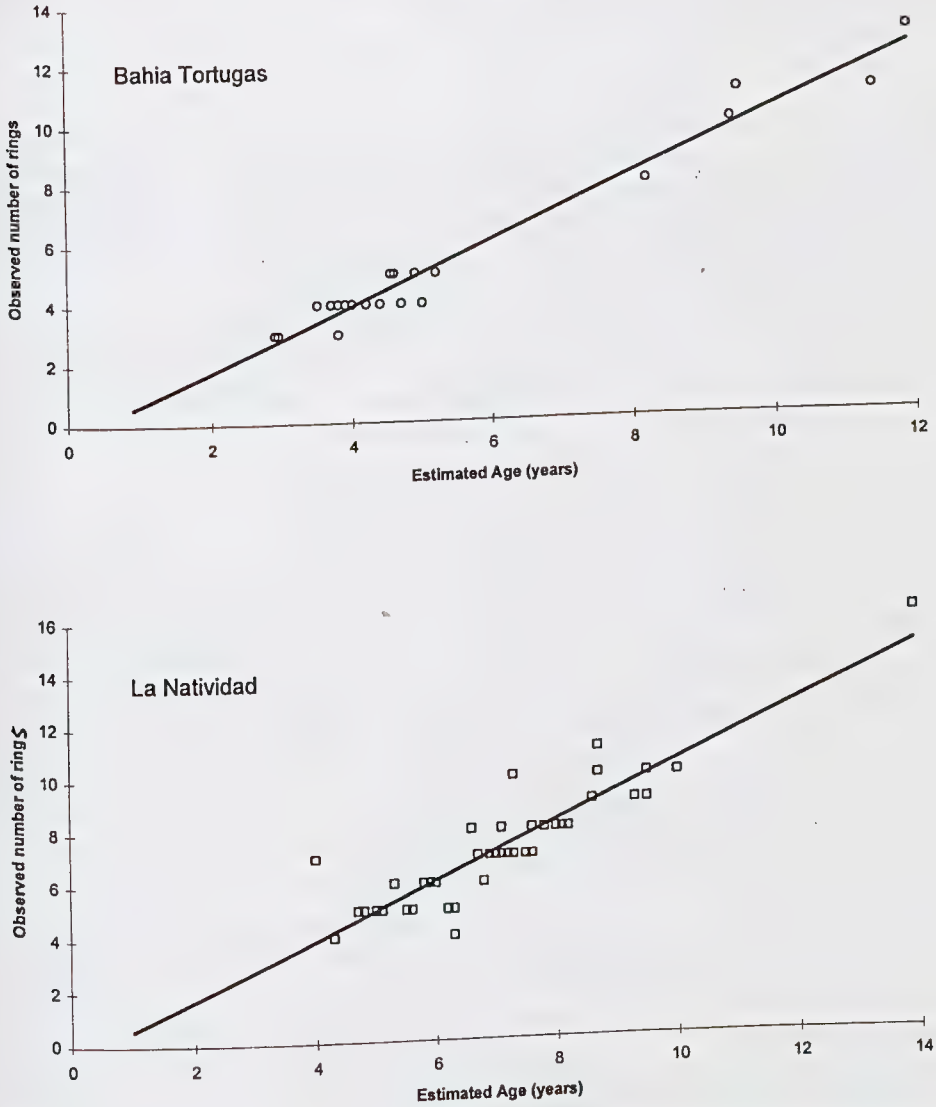

Figure 3. Plots of observed number of dark rings vs estimated age (yrs) at La Natividad and Bahia Tortugas.

Table 2. Comparison of parameters of von Bertalanffy growth model fitted to length-increment (L-I) and age-length (A-L) data for *H. fulgens* at (1) Bahia Tortugas and (2) La Natividad in this paper and previously published work.

Site	Date	N	t_o (s.e)	K(s.e)	L_∞ (s.e)
(1)	L-I (this paper)	24	n.a.	0.37 (0.04)	190.2 (5.9)
(1)	L-I (Shepherd et al. 1991)	32	n.a.	0.38 (0.04)	183.1 (6.1)
(1)	L-I (Turrubiates and Castro 1992)	38	n.a.	0.44	177*
(1)	A-L (this paper)	65	0.36 (0.16)	0.36 (0.06)	182.6 (8.5)
(1)	A-L (Turrubiates and Castro 1992)	453	0.43	0.24	175.3
(2)	L-I (this paper)	49	n.a.	0.35 (0.02)	187.1 (3.1)
(2)	A-L (this paper)	159**	0.78 (0.41)	0.29 (0.07)	179.6 (7.0)

* Turrubiates and Castro (1992) fixed L_∞ at 177 mm and then derived K.
** includes a 1985 sample from La Natividad

Growth rates

Growth parameters derived from length-increment data (El Niño growth checks) and age-length data are given in Table 2 and compared with published values mostly analysed in the same way. The growth parameters for Bahia Tortugas are very close (with overlapping standard errors) to those published by Shepherd *et al.*(1991) but higher than those of Turrubiates and Castro (1992) for age-length data. There are no previously published growth parameters for La Natividad but the two methods produce parameters whose standard errors also overlap.

To test the effect of loss of rings from shell erosion on our estimates of the growth parameters we took an extreme case and assumed that every shell >175 mm SL had lost one dark ring, i.e. that age was under-estimated by one year, and then recalculated the growth parameters for the age length data. The parameters were lower by between 0.3–1.2% and in no case significantly so. So we concluded that shell erosion causes no significant bias to estimates of growth parameters.

Discussion

This study confirms some of the findings of Shepherd *et al.* (1995a), gives additional information on the dynamics of fine ring deposition, and most importantly validates Turrubiates and Castro's (1992) method of aging this abalone over its full size range. The average number of rings deposited in the first year (3.5) is slightly less than that (3.9) which Shepherd *et al.*(1995a) found, but the variability is higher than was formerly thought. The change in pattern of fine rings with age means that the technique of Shepherd *et al.* (1995a), while useful for about the first 4–5 years, is not valid for older shells. In such shells counts of the dark rings are the only reliable indicator of age. However, we recommend using both techniques in shells to about 5 years old because of the variability in pigmentation of rings. We also advocate the use of horizontal sections which show the pigmentation more clearly but note that fine rings are sometimes missed in horizontal section although visible in vertical section (Shepherd *et al.* 1995a). So far the only other species of abalone which shows an alternating pattern of fine and dark rings is *H. iris* (unpublished observations). The mean erosion rate of rings (1 ring per 32 mm SL) is very close to that (1 ring per 32.3 mm SL) found by Shepherd *et al.*(1995a) but may be under-estimated for very large shells. The largest size class of shells examined (175–200 mm SL) would on average have lost 3.5 rings which is the mean number of fine rings before the first dark one. So some must have lost the first dark ring as well. In these cases second year rings would have been counted as first year rings so leading to an under-estimate of age. However, this bias seems not to be serious, at least for estimating growth rates, as it had no significant effect on our estimates. The 1982–3 and 1991–2 El Niño events were both intense and persisted for over a year. The event was stronger in the fall of 1983 than of 1982 (Dayton and Tegner

1984; Norton *et al.* 1985), but conversely was stronger in 1991 than in 1992 (Haywood *et al.* 1994). This may explain why growth checks occurred in shells in late 1983, but not in 1982 and in late 1991 but not in 1992. While large temperature changes and loss of algae are of likely importance in producing a growth check (see Shepherd *et al.* 1995b), spawning may also be a factor.

Growth rates

The closeness of our parameter estimates for the growth check data to other published values (Table 2) reinforces the conclusion that the growth checks were laid down consistently at about the same time during the El Niño events.

The discrepancies between growth parameters derived for length-increment data and age-length data are not surprising given that the analyses address different questions (Francis 1988; and see discussion in Day and Fleming 1992). The discrepancy between our parameter estimates and those of Turrubiates and Castro (1992) for the age-length data at Bahia Tortugas (Table 2), (our data being a subset of those of Turrubiates and Castro), may be due to differences in the estimated ages of younger shells. In this study our estimates were better because we counter-checked the age by counting fine rings and so could detect aberrations in the number of dark rings deposited (see Results).

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Post-settlement survival of abalone (*Haliotis iris, H. australis*) in turbulent flows.

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Abstract

An experimental flow tank which reproduces an oscillating flow typical of shallow coastal waters (ca. 2 m.sec^{-1}) was used to experimentally examine the influence of water flow on the post-settlement survival of abalone (*Haliotis iris* and *H. australis*). Experimental inocula of hatchery-reared larvae settled on substrata of contrasting crustose coralline morphology (encrusting vs warty) were monitored during 24 h exposure trials to calm and turbulent flow regimes. Results indicated greater survival of *H. iris* on encrusting coralline surfaces (*Spongites yendoi*) than on warty surfaces (*Mesophyllum printzianum*): a result attributable to differential predation by infaunal polychaete worms. Survival of *H. australis* on both surfaces was much lower than for *H. iris* suggesting interspecific differences in the interaction between surface morphology and turbulence. The results suggest that both *H. iris* and *H. australis* are well adapted to torrential flows in nearshore habitats, but there is an interaction between the physical and biological aspects of the habitat to which these abalone recruit.

Keywords: *Haliotis iris*, *H. australis*, water flow, post-settlement survival, crustose coralline algae

Introduction

Crustose coralline algae are well known for their roles both in the induction of settlement and metamorphosis in abalone (*Haliotis* spp.) and other invertebrate larvae (Morse *et al.* 1979, Morse and Morse 1984, Johnson *et al.* 1991), and in the provision of suitable habitat for post-settlement growth and survival of abalone (Shepherd and Turner 1985, McShane 1995). Subsequent survival and growth on coralline substrata has been variously attributed to the availability of food, such as that provided in surface biofilms and later by the coralline crust itself (Garland *et al.* 1985, Johnson and Sutton 1994).

From the results of experimental studies, we proposed that the survival of post-settlement abalone (*Haliotis iris* Martyn) was influenced by water movement in the near-shore habitat (McShane and Naylor 1995). Furthermore, we suggested that the effects of water movement would be modified by the topographic complexity of coralline substrata. The topographically complex coralline substrata, more likely to afford protection from wave-driven shear forces, are more prevalent in sheltered habitats such as the understories of kelp forests or on reefs in deep water (McShane 1996). Conversely, encrusting corallines, which we expect to give poor protection to post-settlement individuals from water movement, are prevalent in open shallow reef habitat (McShane 1996).

Here, we show how a simple, inexpensive flow tank can be constructed to realistically simulate water flows representative of near-shore subtidal reef habitats. We use the flow tank to experimentally test the hypothesis that the effect of water movement on the post-settlement survival of *H. iris* and *Haliotis australis* Gmelin depends on the topography of the coralline substrata to which they recruit.

Materials and methods

Experimental flow tanks

Water movement was created within two flow tanks (Fig. 1). The moulded fibreglass tanks were modified by adding a central oval partition to allow directional water flow. The slow flow treatment was created by introducing a laminar flow into each end of the tank at low velocity. The high flow treatment was created by injecting two jets of high velocity water at each end of the tank, and supplementing this by adding the contents of a 20-l bucket of water to each end every 10 seconds. The buckets were suspended on wooden frames on an off center axis 1 m above the flow tank, and had a 0.5 kg weight secured to their outside bottom edge (Fig. 1). The two buckets were constantly filled with fresh seawater and once three quarters full, tipped and discharged. They then swung back

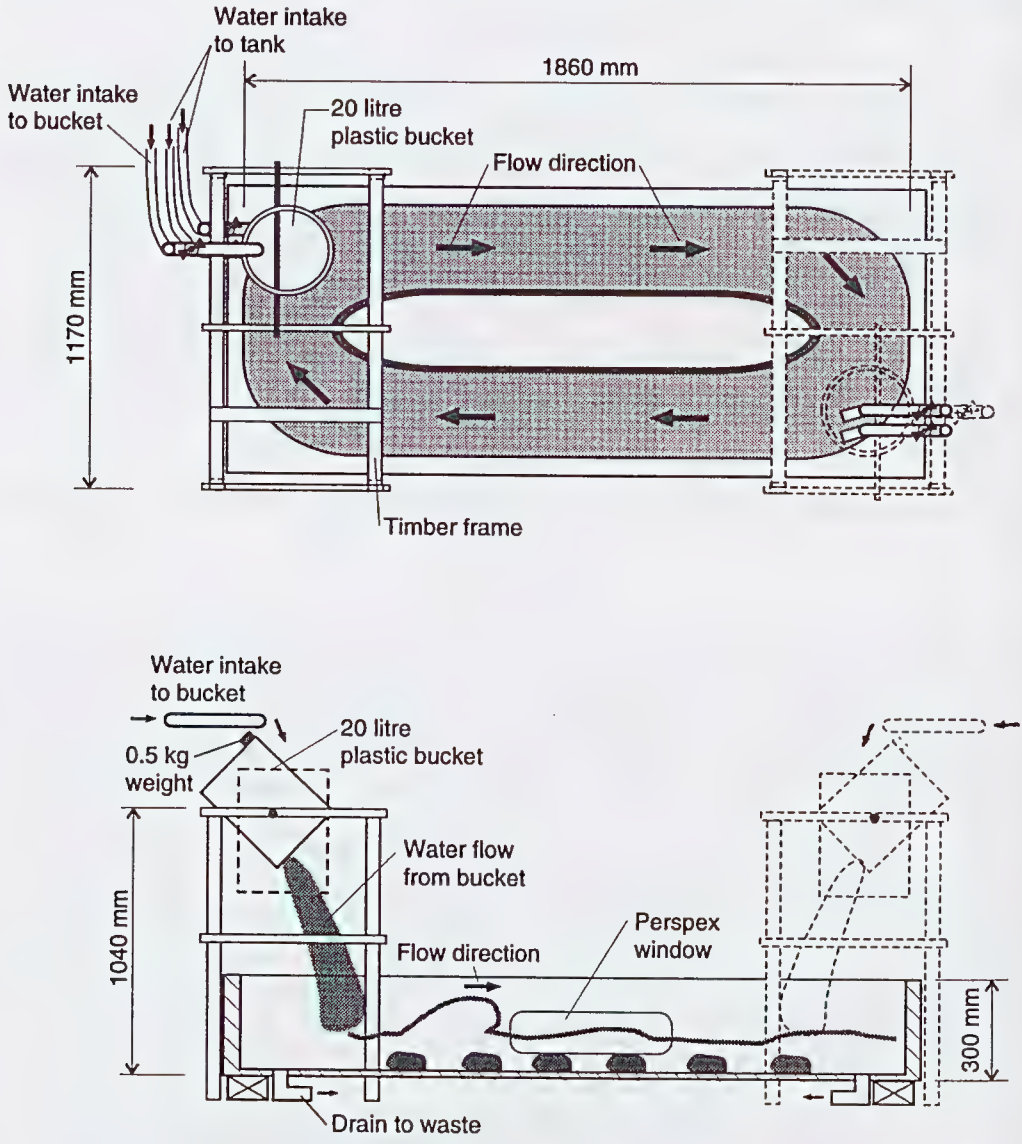

Figure 1. Schematic diagram of the flow tank used in post-settlement survival experiments.

to their original vertical position and began to fill. This simulated the oscillating flow typical of nearshore shallow habitats.

Water flow was measured directly by timing the passage of fluorescent dye over 1 metre. Five replicate measurements were made for each flow regime.

Experimental design

Boulders of similar shape and size (about 200mm in diameter) were obtained from Reef Bay, Wellington, New Zealand. This generally sheltered location provides small boulder/cobble habitat on a gently sloping reef to about 8 m depth (McShane and Naylor 1995). Boulders were chosen the upper surfaces of which were coated with encrusting coralline (*Spongites yendoi*) and warty coralline (*Mesophyllum printzianum*). These coralline surfaces provided contrasting morphology (encrusting vs warty) following the terminology described by (Woelkerling *et al.* 1993).

Larvae were hatchery-reared according to the methods described in Tong and Moss (1992) and Moss and Tong (1992). Inoculation occurred 9 days after fertilisation at which time the larvae had developed 8 rows of chitonised teeth. Post-settlement survival of *H. iris* is greatly increased in larvae with 8 or more rows of teeth (Moss and Tong 1992). Before inoculation, boulders were placed in individual 4-l containers each provided with a jet of running seawater. Separate containers were used to minimise the effects of physical gradients which commonly occur across larger aquaria and which may have resulted in variable larval settlement between boulders. Immediately before inoculation, and for 24 h after, the water supply to the containers was stopped to prevent the loss of larvae by overflow. Approximately 2000 larvae were added to each container.

The experiment was designed to conform to a two factor (morphology, water movement) split-plot analysis of variance (Sokal and Rohlf 1981). There were two levels each of morphology (encrusting and warty) for each tank (calm and turbulent). There were 5 replicates per treatment. All factors were considered fixed and variances were tested for homogeneity with Cochran's test (Underwood 1981). Each species was tested separately.

Experimental substrata were placed at random in two identical flow tanks; one subject to the oscillating turbulent flow (ca. 2 $m.sec^{-1}$), and the other subject to the calm uni-directional flow (ca. 0.1 $m.sec^{-1}$). Boulders with either encrusting or warty substrata were placed at random approximately 10 cm apart in the center of the flow line of each tank. Recruits were removed after 24 h from the experimental substrata by soaking in 5% buffered formalin before being counted and measured (to the nearest mm) under a dissecting microscope.

Results

Mean flow rates (s.e.) in the experimental tanks were measured to be 0.10 (0.01) $m.sec^{-1}$ for the calm flow regime, and 1.83 (0.10) $m.sec^{-1}$ for the turbulent flow regime. A mean residual flow of 0.53 (0.02) $m.sec^{-1}$ was maintained in the turbulent flow tank between discharges.

After 24 hours the survival of *H. iris* was greater on encrusting coralline algae than on warty surfaces (Table 1), but there was no significant variation attributable to water flow (Table 2). In general, post-settlement survival of *H. australis* was much less than *H. iris* (Table 1). Although survival of *H. australis* was apparently greater on warty coralline growth forms, these differences were non-significant (Table 2).

Table 1. Survival (numbers of individuals) of *Haliotis iris* and *H. australis* on encrusting and warty coralline surfaces under contrasting flow regimes. Data are means (*s.e.*).

	Haliotis iris		*Haliotis australis*	
	Encrusting	Warty	Encrusting	Warty
Calm	185 (48)	120 (45)	5 (3)	17 (7)
Turbulent	224 (50)	62 (23)	3 (1)	6 (4)

Table 2. Effects of turbulence and coralline morphology on the survival of post-settlement abalone *Haliotis iris* and *H. australis*. Split-plot ANOVAs are shown for the dependent variable (numbers surviving after 24 h).

Haliotis iris after 24 h.
split-plot ANOVA n = 5; Cochrans untransformed data n.s. (0.389)

Source	SS	df	MS	*F*	*P*
Morphology	75351.7	4	18837.9	6.13	< 0.01
Turbulence	135.2	1	135.2	0.02	n.s.
M x T	14191.3	4	3547.8	1.16	n.s.

Haliotis australis after 24 h.
split-plot ANOVA n = 5; Cochrans untransformed data n.s. (0.693)

Source	SS	df	MS	*F*	*P*
Morphology	326.7	4	81.7	1.04	n.s.
Turbulence	186.1	1	186.1	2.05	n.s.
M x T	501.7	4	125.4	1.6	n.s.

Discussion

We attempted to experimentally mimic the water flows typical of the nearshore subtidal habitat of abalone (*Haliotis iris* and *H. australis*). The oscillating horizontal flows of about 2 m.sec^{-1} reproduced in our flow tank were approximately twice the maxima created by a 1 m unbreaking wave in 2 m of water (from the simplified equations for oscillating wave-induced flows in shallow water; U.S. Army Corps of Engineers 1984) and may be considered torrential (Vogel 1994). However, the water flows in the flow tank may be considerably modified by the experimental substrata just as nearshore water movement is modified by the topography of subtidal reef habitats. Cogent descriptions of the nature of drag and lift forces associated with various substrata are given by Denny (1988) and Vogel (1994). Texts such as these show that the quantification of such forces at the boundary layers of heterogeneous substrata is not simple. For example, the rough texture of the warty *Mesophyllum* substratum may induce a transition from a high drag boundary layer state to a low drag turbulent boundary layer state in the high flow environment (Denny 1988). However, Denny (1989) suggested that such mechanisms to reduce drag may not be available to nearshore benthic organisms. Attempts at visualising flow patterns associated with the two types of experimental substrata with dye streams were not successful as the considerable turbulence masked any obvious differences.

The water turbulence created in our experiment was insufficient to cause significant variation in the post-settlement survival of abalone. However, breaking waves (> 1.5 m in 2 m of water) would create horizontal flows much greater than the 2 m.sec^{-1} reproduced in our flow tank. It is possible that infrequent exposure to strong flows caused by breaking waves in nearshore habitat could cause dislodgement of recruits from hard substrata (cf. Denny 1988). The adhesion of recruits in the torrential flows examined in our study may be facilitated by strong pedal adhesion facilitated by secreted mucus such as that shown for limpets by Denny (1989).

Clearly, in living in subtidal habitats of extreme exposure, *H. iris* and *H. australis* are well adapted to strong water flows. Experimental trials revealed that post-settlement individuals were much more resistant to removal by suction samplers (McShane and Smith 1988) than similar-sized *H. rubra*. Post-settlement individuals of *H. rubra* which recruit to encrusting coralline surfaces of the understory of macroalgal forests (McShane *et al.* 1988) were all removed by suction whereas only 59% (s.e. 10% for 5 trials) of *H. iris* recruits were removed (McShane and Naylor unpubl. data). Although further studies are necessary, these preliminary results suggest an interspecific difference in pedal adhesion and resistance to lift forces for post-settlement abalone. Suggestions by Shepherd and Daume (1996) that warty coralline algae afforded a poor surface for pedal adhesion by post-settlement abalone were not supported from the results of our study.

The post-settlement survival of *H. iris* was much greater than that of *H. australis*. The contrary result of greater survival of recruits on encrusting than on warty substratum for *H. iris* can be explained by differential predation by infaunal polychaetes (Naylor and McShane 1997). However, as both species were equally vulnerable to predation, other factors were responsible for the interspecific difference in survival. We had previously observed recruits of *H. australis* re-entering the water column up to several days after settlement. In contrast, individuals of *H. iris* adopt a benthic existence after settlement and were never observed re-entering the water column. Thus, *H. australis* may be expected to be more vulnerable to turbulence than *H. iris*. Although the results suggested greater survival on the warty substratum than on the encrusting coralline substratum, the generally low survival rates masked any difference. Our results and observations suggest great differences in the early life histories of these congeneric abalone.

Physical protection from lift and drag forces induced by strong water flows in nearshore habitats may yet prove to be important to survival of post settlement abalone. However, further studies are required to assess the complex physical and biological interactions taking place within the microenvironment associated with the surface of crustose coralline algae.

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Studies on southern Australian abalone (genus *Haliotis*) XVII. A chronology of *H. laevigata*

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Abstract

The abalone *Haliotis laevigata* deposits 2 rings yr^{-1} in the spire during the first 4–6 years of life at 11 sites in southern Australia. At two sites it deposits 3 rings yr^{-1}, and at another, 3 rings yr^{-1} for the first 3 years and 2 yr^{-1} in the next 3 years. These rates of ring deposition appear to be dependent on the growth rate; they are not exact for every individual due to individual variation in growth but appear to be a statistical property of the population. The tendency for an integer number of rings to be deposited annually may be related to exogenous cues. Hence at one site where 3 rings yr^{-1} are laid down they are deposited in about June, September and December each year, corresponding to winter, the spawning season and summer. Long term tag recaptures suggest that these rates of deposition persist in older shells but better validation is required. Erosion of the shell causes the loss of rings, and the attack of muricid and polydorid borers causes the deposition of adventitious rings as well as pigmented rings, called brown rings. These complications need to be considered in estimating the age of a shell from ring counts.

Key words: *Haliotis laevigata*, growth, shell aging, chronology, borers, shell erosion, brown rings.

Introduction

The ability to age an abalone shell provides a powerful tool for stock assessment of abalone fisheries because mortality and growth rates can be readily derived without long and costly field experiments. Hayashi (1955) discovered rings in the abalone shell (which are of simple prismatic structure and have been variously described since as growth lines, dark layers, conchiolin layers or prismatic layers) and proposed their use in aging the shell. Muñoz-Lopez (1976) first applied the technique to Mexican species of abalone and it has now been successfully applied to six species (Prince *et al.* 1988, Erasmus *et al.* 1994, Shepherd *et al.*1995a,b; Shepherd and Turrubiates 1997, Shepherd and Avalos-Borja 1997, Shepherd and Huchette 1997).

The greenlip abalone, *Haliotis laevigata* Donovan, is fished commercially in southern Australia and lays down non-pigmented rings in the spire, clearly visible in horizontal shell sections. Occasionally, brown rings, so called because they are darkly pigmented, are also laid down. In this paper we use several independent techniques to estimate the rate of deposition of rings in the spire of the shell of this abalone from a number of sites which were chosen to span the main fishing areas in a range of habitats in southern Australia. We also examine two problems, parasitism of the shell and shell erosion, that confound shell aging in order to determine their effect and significance in aging studies.

Materials and methods

Data collection

Shell samples of *H. laevigata* were collected from 14 study sites (Fig. 1) where the growth rate of

this abalone had been previously studied (Shepherd and Hearn 1983, Shepherd 1988, Shepherd *et al.* 1992, Wells and Mulvay 1995, unpublished data). Using the techniques described in detail by Shepherd *et al.* (1995b) we took horizontal sections by rasping the spire to expose the rings and counted them under a low power binocular microscope. We discarded shells heavily bored by boring organisms as they were unreadable. For the Taylor Island site we also examined monthly collections of 10–15 adult shells in order to estimate the time of deposition of the rings. The shells were sectioned at the spire as described above to expose the layer of shell (whether a ring or nacre) most recently laid down. Two additional samples of shells grown in culture were examined. The parent broodstock of both samples came from Taylor Island. One sample was grown in a hatchery for 25 months at high final densities of > 200 m^{-2}, and the other was grown at low densities (<20 m^{-2}) for 20 months. We examined samples of shells taken by commercial divers from Hopkins Island and Cathedral Rock in Thorny Passage to determine the relation between the deposition of brown rings

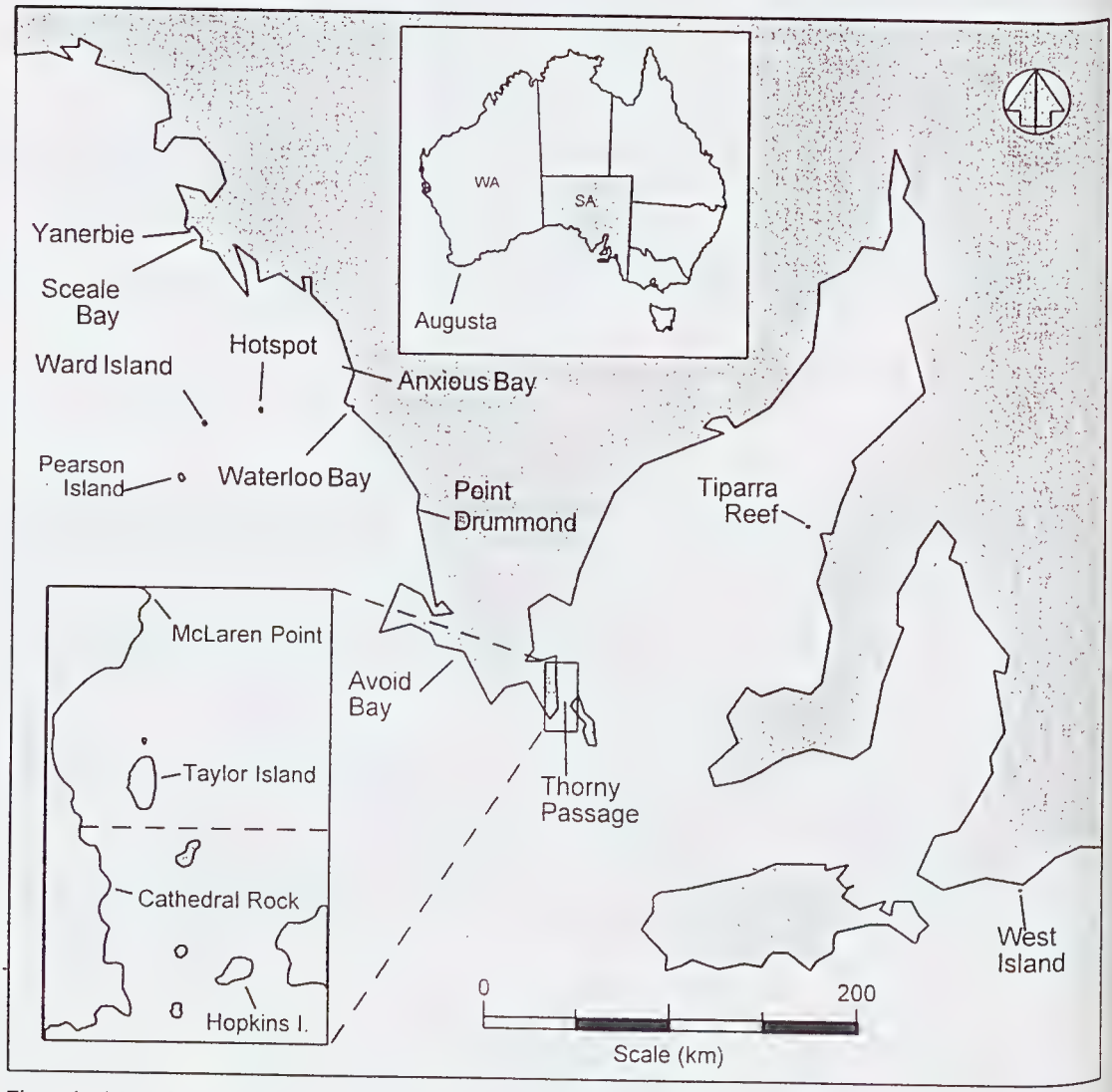

Figure 1. Map of South Australia with insets showing study sites.

(described by Shepherd and Huchette 1997) and drilling of the shell by the muricid snail *Haustrum baileyanum* (Thomas and Day 1995) and boring of the shell by polydorid polychaetes (Shepherd 1973). We categorised shells as slightly bored when < 50% of the sectioned surface area at the spire (about 1 cm^2) was intensely perforated by borers and moderately bored when > 50% of the area was infested. Our third category of heavily bored shells was the unreadable ones. We then scored shells for the presence or absence of brown rings in 4 categories of shell: those (a) slightly or (b) moderately bored by polychaetes and (c) with or (d) without muricid drill holes. The presence of drill holes is most easily seen by examining the ventral nacreous surface of the shell where a half pearl of nacreous material is always deposited at the site of the hole; this can then be confirmed by dorsal examination of the shell. To estimate the effect of erosion of the shell and of polychaete boring on the number of extant rings we examined a sample of shells from Sceale Bay where both effects were conspicuous. In eroded shells, commonly known as "shinybacks", the periostracum is worn away exposing the nacreous layer. We compared the net rate of deposition of rings with size in samples of shinybacks, uneroded shells and bored shells in the size range 100–150 mm shell length (SL).

Statistics

The growth rate of *H. laevigata* is linear with age for the first 4–6 years according to site and thereafter is curvilinear. The linear phase can be fitted by a linear regression and the curvilinear phase by a von Bertalanffy growth curve (Shepherd and Hearn 1983; Shepherd 1988; Shepherd *et al.* 1992). Shell length is a good predictor of age in the linear phase but not in the curvilinear phase because of the dependence between the growth parameters K and L. This can be seen by looking at the derivative with respect to time of the von Bertalanffy equation:

$$L_t = L_\infty (1\text{-}e^{-kt})$$

At t=0, $L' = L_\infty K$ i.e., the same slope will be obtained with different values of L_∞ and K. Another difficulty is that during the linear phase the growth rate of individuals varies and the variance of the mean growth rate increases, apparently linearly with length (see discussion in Day and Fleming

Table 1. The sites studied, showing the methods used to estimate growth rates, the length range to which they apply, and the mean growth rates (mm yr^{-1}) during the linear growth phase with authority. Abbreviations: s.e. = standard error; T = tagging studies; CS = growth of cohorts from snapshot data sets.

Site	Method	Length range (mm)	Growth rate (s.e.)	Authority
West Island	T	25–110	20.3 (0.4)	Shepherd (1988)
Tiparra Reef	CS	48–102	20.9 (0.7)	Triantafillos (1994) – 1990 data
McLaren Point	CS	20–140	23.6 (1.1)	Shepherd *et al.* (1992)
Taylor Island	CS,T	15–145	39.6 (0.9)	Shepherd *et al.* (1992)
Avoid Bay	T	45–115	19.7 (2.4)	Unpublished 1987 data
	CS		18.1 (0.5)	Unpublished 1995 data
Pt Drummond	CS	10–110	24.7 (2.2)	Huchette (1995)
Pearson Island	CS	50–125	16.9 (0.3)	Huchette (1995)
Ward Island	T	60–125	25.7 (1.5)	Shepherd *et al.* (1992)
	T		21.3 (2.1)	Revised estimate with more data
	CS		19.4 (2.3)	Unpublished 1986 data
Hotspot	CS	50–120	20.7 (2.6)	Huchette (1995)
Waterloo Bay	CS,T	50–120	20.8 (0.3)	Shepherd and Silveira (in prep.) – 1997 data
Anxious Bay	CS,T	25–95	20.4 (1.5)	Shepherd *et al.* (1992)
Sceale Bay	CS,T	45–110	20.4 (1.8)	Shepherd *et al.*(1992)
Yanerbie	CS,T	15–110	15.3 (0.9)	Shepherd *et al.* (1992)
Augusta,W.A.	T	20–100	31.6 (1.8)	Wells and Mulvay (1995)

1992). This does not invalidate the use of length as a predictor of age but it requires that sample sizes be larger to reduce the variance of the estimated regression slopes. For each site we first obtained a linear regression equation expressing the relation between length and age during the linear phase of growth (Table 1). We used published estimates of the growth rate, or more recent unpublished estimates. For two sites we give more than one estimate, because different methods produced slightly different growth rates or, as at Ward Island, we have more data. In the case of Waterloo Bay, more recent studies suggest a decline in the growth rate possibly due to selective fishing (Shepherd and Silveira in prep.), so we used the most recent estimates. We then regressed the number of rings vs shell length for each site by least squares analysis. By substituting the relation between length and age (Table 1) in these regression equations we derived estimates of the number of rings laid down annually. Standard errors of multiplicands were calculated with formulae from Green and Margerison (1977). The data for McLaren Point showed a change in slope so we fitted a broken stick model i.e., two linear regressions with a visually selected break point. No other data set gave hint of a change in slope.

The statistical power of the regressions to detect a 25% change in slope, at a significance level of á=0.05 at the given sample sizes, was calculated with a Statgraphics computer package (Anon. 1986). While an effect size of 50% should be enough to discriminate between deposition rates of integer numbers of rings yr^{-1} we conservatively chose a 25% level.

In the analysis of the mark-recapture data we used the regression equation derived for the site in question (Table 2) to estimate the number of rings laid down at the date of tagging. This was valid because the recaptured abalone were all marked during the linear phase of growth. We counted the number of rings at the spire at the date of recapture and, by deduction, an estimate of the number of rings laid down during the period at liberty.

Table 2. Regression equations of number of rings (R) vs length in mm (L) for *H. laevigata* at 14 sites, with estimates of the number of rings deposited annually and in the first year. N = the number of shells examined . Power is the power to detect a 25% change in the slope at the given sample size. s.e. = standard error.

Site	N	Regression equation	r^2	No. rings yr^{-1} (s.e.)	No.rings in in 1st yr	Power
West Island	81	R = 0.97 + 0.092L	0.343	1.87 (0.61)	2.8	0.49
Tiparra Reef	32	R = -0.63 + 0.109L	0.779	2.28 (0.22)	1.2	0.79
McLaren Point	63	(a)R = -2.07 + 0.125L	0.772	2.96 (0.25)	0.8	0.99
	88	(b)R = 2.25 + 0.081L	0.510	1.90 (0.22)		0.73
Taylor Island	150	R = 0.30 + 0.079L	0.750	3.14 (0.17)	1.5	0.99
Avoid Bay	60	R = 0.33 + 0.090L	0.793	(1) 1.77 (0.28)	2.0	0.99
				(2) 1.62(0.17)	2.2	
Pt Drummond	16	R = 1.44 + 0.091L	0.973	2.22 (0.17)	3.3	0.99
Pearson Island	32	R = -1.64 + 0.118L	0.707	1.99 (0.23)	0.4	0.72
Ward Island	58	R = 1.58 + 0.094L	0.628	(1) 2.41(0.27)	2.3	0.99
				(2) 2.00 (0.28)	2.8	
				(3) 1.82 (0.28)	3.1	
Hotspot	51	R = 0.10 + 0.102L	0.472	1.98 (0.15)	1.9	0.52
Waterloo Bay	60	R = 0.002 + 0.107L	0.764	2.23 (0.17)	2.7	0.95
Anxious Bay	71	R = 0.67 + 0.111L	0.848	2.26 (0.22)	2.1	0.99
Sceale Bay	66	R = 1.42 + 0.089L	0.628	1.82 (0.24)	1.5	0.8
Yanerbie	62	R = -0.37 + 0.124L	0.813	1.90 (0.16)	1.0	0.99
Augusta, W.A.	41	R = -0.38 + 0.099L	0.672	3.14 (0.39)	2.8	0.96

The multiple values for the number of rings yr^{-1} given for Avoid Bay and Ward Island refer to the multiple growth rates given for those sites in Table 1. The two regression equations for McLaren Point relate to the two parts of the broken stick model.

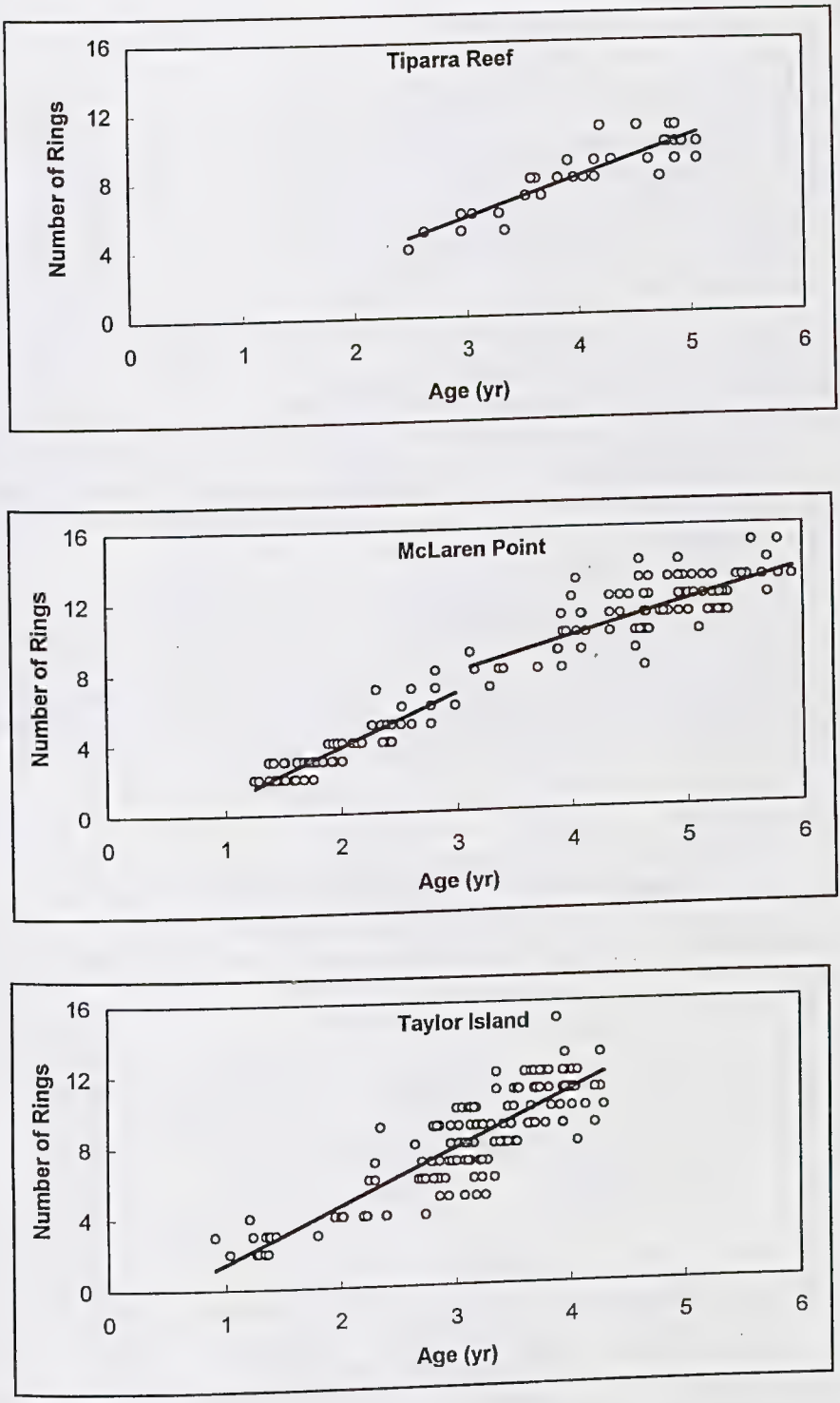

Figure 2. Plots of number of rings vs shell length for three sites showing different patterns of ring deposition: Tiparra Reef (2 rings yr^{-1}), Taylor Island (3 rings yr^{-1}) and McLaren Point (3 then 2 rings yr^{-1}).

Results

Deposition rates

The sites studied, with estimates of the growth rate of *H. laevigata* and authority, are given in Table 1. The regression equations relating the number of rings to shell length, with estimates of the number of rings deposited in the first and subsequent years, are given in Table 2. Plots for three sites illustrating the different patterns of deposition are shown in Fig. 2. The number of rings laid down did not differ significantly from 2 yr^{-1} at 11 of the sites and 3 yr^{-1} at two sites. At one other site (McLaren Point) the rate of deposition changed from 3 to 2 yr^{-1} at about three years of age. The change in slope of the regressions (Fig.2) was significant ($t=8.0$; $P<0.001$). Although none of the three estimates of rates of ring deposition for Ward Island differed significantly from 2, the last two, based on more or more recent data, are probably more accurate. Statistical power (Table 2) was adequate (0.7-0.99) to detect a change of 25% in the slope of all the regressions except at West Island and Hotspot, but even at these sites it was adequate (>0.9) to detect a 50% change.

Next we examined the probability of deposition of the nearest alternative integer number of rings yr^{-1} for each site. For example, at West Island our estimate was 1.87 rings yr^{-1} (Table 2), so we examined the hypothesis of 1 ring yr^{-1}, and rejected it ($t=2.5$; $P<0.02$). Similarly, we rejected the hypotheses of the nearest alternative integer for every other site (in every case $3.1<t<6.7$ and $P<0.001$ except for Ward Island where the nearest alternative for 2.4 rings yr^{-1} i.e. 3 rings yr^{-1} was rejected with $t=2.5$, $P<0.05$).

The number of rings laid down in the first year appeared to vary from 1 to 3 according to site (Table 2). However, little confidence can be placed in any of them. The standard errors (not shown) are >1 and in any case the estimates are extrapolations beyond the range of the data. The mean growth rates of the two samples of shells of *H. laevigata* grown in culture differed significantly ($t=8.0$; $P< 0.001$) from each other (Fig. 3), and within each sample there was wide variability in growth rate. Although the mean number of rings deposited did not differ significantly from 3 yr^{-1} for either sample (Table 3), individuals deposited a variable number of rings ranging from 2.3–4.3 yr^{-1}

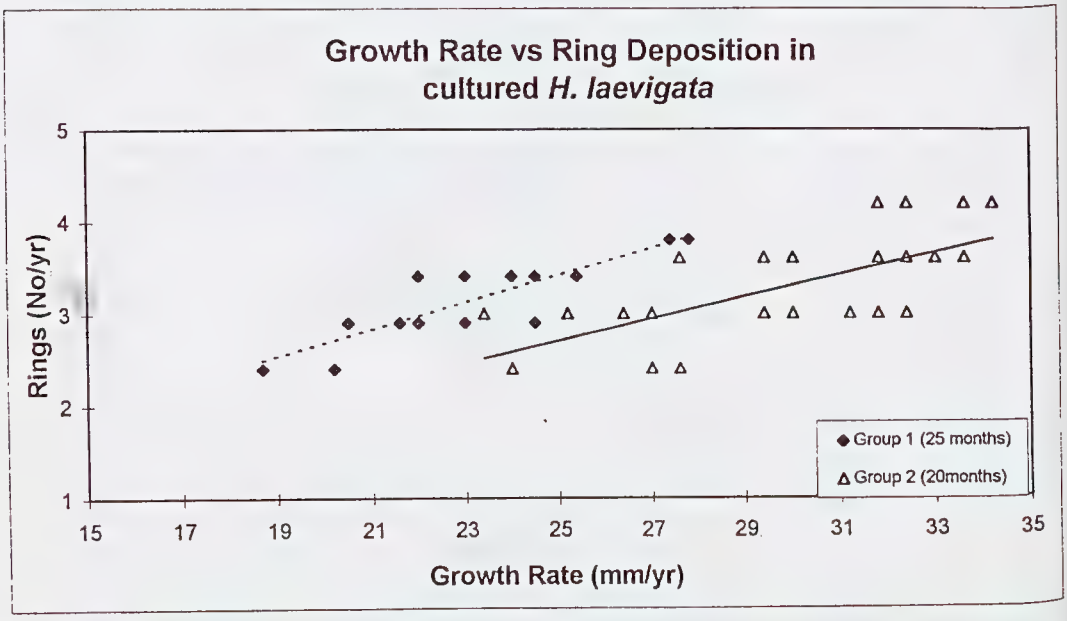

Figure 3. Plots of deposition rate vs growth rate for two samples of shells grown in culture at different growth rates.

Table 3. Rates of deposition of rings in shells of *H. laevigata* grown in culture with regression equations of number of rings (R) vs growth rate (GR) in mm yr^{-1}. N = sample size. Both regression slopes were significant: (1) $P<0.001$; (2) $P<0.05$.

Sample	Period (mths)	N	Shell lengths (mm)	No. of rings yr^{-1}	Regression	r^2
(1)	25	19	39–58	3.19 (0.09)	R=-0.26+0.15GR	0.719
(2)	20	29	39–57	3.29 (0.10)	R=-0.27+0.12GR	0.460

(Fig.3). The fractions of rings result from adjustments for the non-integer number of years at liberty. Among individuals in each sample a regression of the number of rings yr^{-1} vs the growth rate was significant (Table 3, Fig. 3).

In the monthly samples of adult shells (120–160 mm SL) from Taylor Island examined for evidence of time of deposition of rings, a plot of the proportion of the shells with a ring newly laid down vs time (Fig. 4) shows that 90–100% of the samples taken in June, September and December had just deposited a ring. This implies that adult shells, as well as juveniles, deposit about 3 rings annually at this site.

Tagging data

The mark-recapture data for 73 shells (Table 4) show variability in the number of rings laid down between and within sites. Yet 63 of the shells (variously grouped to illustrate points discussed) deposited, on average, very close to 2 or 3 rings annually according to site. At West Island and Tiparra Reef the mean number is 1.9–2.1 for individuals at liberty from 2 to 15 years. At Taylor Island some were marked *in situ* and others removed from the water for tagging in separate experiments. There were no significant differences in the number of rings laid down by the two

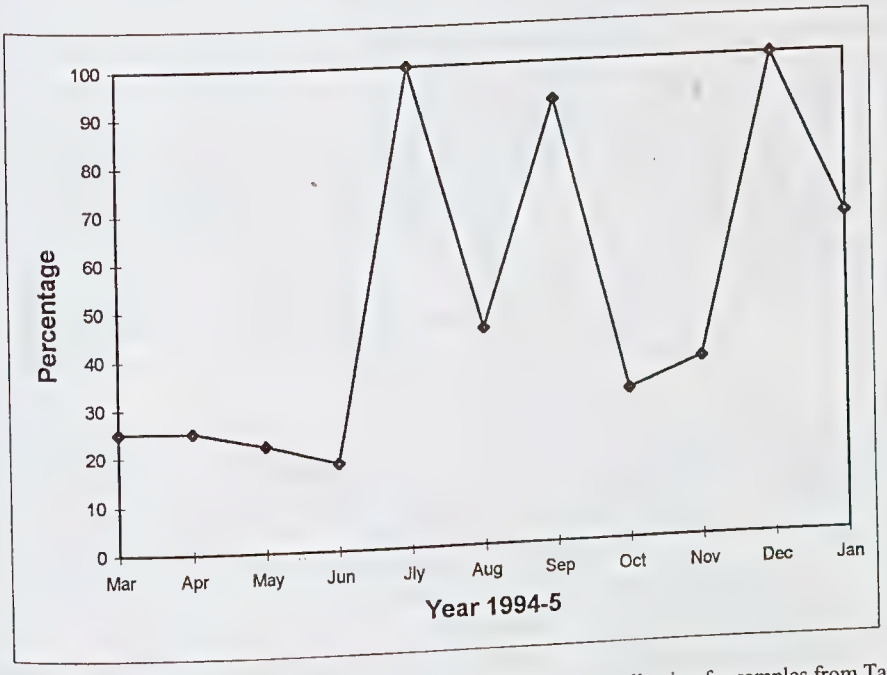

Figure 4. Plot of proportion of shells with a ring newly laid down vs month of collection for samples from Taylor Island.

Table 4. Summary of mark-recapture data for *H. laevigata* at various sites with the estimated number of rings deposited per annum during the period at liberty. Data for West Island, Taylor Island and Waterloo Bay are shown in several groups to illustrate features discussed in the text. N = sample size; Age = estimated age at tagging; Period = mean period at liberty in years (with standard error).

Site	N	Age (s.e.)	Period (yrs)(s.e.)	Rings yr^{-1} (s.e.)	Comments
West I.	4	3.1 (0.03)	2.1 (0.6)	1.8 (0.8)	
	2	2.3 (0.4)	2.0 (1.0)	0	Did not grow
	1	3.8	15.3	1.9	
McLaren Point	1	5.5	2.3	0.4	Grew very slowly
Taylor I.	4	2.7 (0.2)	2.1 (0)	4.7 (0.7)	Heavily bored by polydorids
	7	2.4 (0.3)	3.6 (0.4)	2.7 (0.3)	Tagged out of water
	18	2.4 (0.1)	2.1 (0)	2.9 (0.2)	Tagged *in situ*
	19	2.3 (0.1)	1.3 (0)	2.5 (0.2)	A mean of 3.3 rings laid down over 16 mths
Avoid Bay	1	3.9	5.7	2.1	
Waterloo Bay	7	3.9	1.0 (0)	2.0 (0.3)	
	1	2.9	8.3	1.5	At a site of stunted growth
	1	3.1	8.3	0.9	Shell badly eroded
Anxious Bay	1	2.6	2.5	1.1	At a site of stunted growth
Tiparra Reef	6	5.1 (0.1)	2.0 (0.2)	2.1 (0.4)	Tagged *in situ*

groups (t=0.5; n.s.). A group at liberty for 16 months laid down a mean of 3.3 rings but marking was done in March and no rings are expected to be laid down until about June (Fig. 4) so the uncorrected number (3.3) is probably a better estimate of the mean annual deposition rate for this group. This example shows that where rings are deposited at a specific time of the year then mark-recapture data for individuals at liberty for a non-integer number of years, when adjusted to annual periods, can under- or over-estimate the deposition rate. Four shells recaptured from Taylor I. were parasitised by polydorid parasites. These shells laid down significantly more rings than others marked at the same time and at liberty for the same period (t=2.7; P<0.02). This could not have been due to their different growth rate because it was slightly, but not significantly, faster than that of the non-parasitised shells. Further, 12.5% of the shells recaptured at Taylor Island showed the deposition of a brown ring at the location in the shell structure where a ring would be expected if it had been laid down at the time of marking.

Six marked individuals, which grew very slowly or not at all, deposited fewer rings than expected. The conspicuously eroded shell (Table 4) may have deposited fewer rings or, more likely, lost some.

Table 5. Relation between the presence of brown rings in the shell and the effect of two kinds of parasites, drilling by *Haustrum* and boring by polychaetes, in *H. laevigata* at Hopkins Island.

Shell characteristics	Brown rings absent	Brown rings present
neither drilled nor bored	58	5
not drilled, but bored	3	30
drilled but not bored	18	46
both drilled and bored	2	22

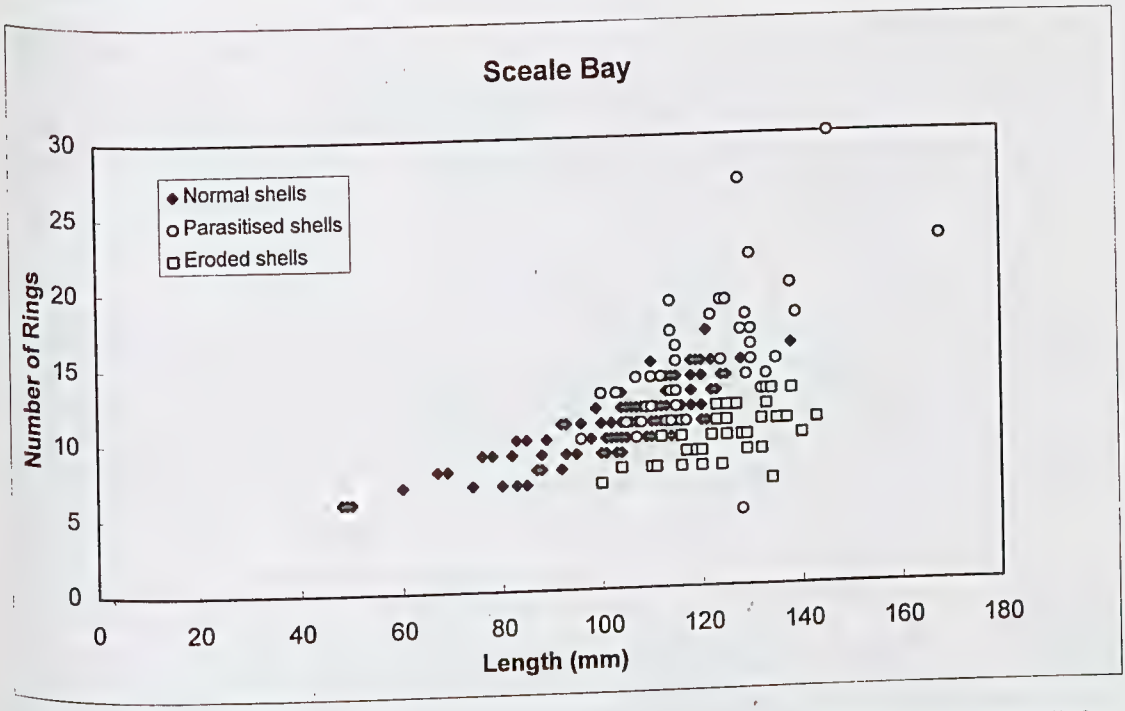

Figure 5. Plot of the number of rings vs shell length for normal, eroded and parasitised shells for a sample of shells from Sceale Bay.

Shell erosion and effect of parasites

At Sceale Bay shells with epizoic limpets were generally eroded whereas shells without these epizoites were overgrown with epizoic algae and were not eroded. Other shells also without these epizoites were bored by polychaetes. A plot of the number of rings vs shell length for a sample of 182 shells (Fig. 5) classified in 3 classes of shell shows that over the size range 45–110 mm SL the number of rings increases linearly with length; the regression equation (Table 2) indicates a deposition rate of about 2 rings yr^{-1}. In larger shells those that are parasitised have more rings than normal shells, and those that are eroded have less. Comparison of regression slopes between the three classes of shells has little meaning because the ranges of the length values, and probably the growth rates, differ between them.

Next we examined the question whether brown rings were caused by parasitic attack in the shell sample from Hopkins I. In all, 56% of the sample had one or more brown rings in the spire although in many cases these were very fine and short and similar to those classified as false rings by Shepherd *et al.* (1995b). The data (Table 5) show that brown rings seldom occur in shells which have not been attacked by parasites and are more likely to be present after parasitic attack. In the 5 examples of brown rings present in unbored and undrilled shells, three of them had prominent growth checks suggesting that some external event had severely affected growth. First we tested the null hypothesis that the presence of brown rings was independent of drilling *and* boring and rejected it ($\chi^2_4 = 247$; $P<0.001$). Then we tested the further null hypotheses that the presence of brown rings was independent of (a) muricid drilling of the shell in the absence of boring by polychaetes, and (b) boring by polychaetes in the absence of muricid drilling. We rejected both hypotheses (for drilling $\chi^2_2 = 64.6$; $P<0.001$: for boring $\chi^2_2 = 53.9$; $P<0.001$) and concluded that both kinds of parasites independently of each other provoke the deposition of a brown ring by the abalone. The reason why brown rings are more often absent in drilled shells (28%) than in bored shells (9%) may be because we scored for drilling over the whole of the shell but for boring only in the region of the

spire. We noticed that brown rings were larger and more likely to be present when drilling occurred near the spire than when it occurred over the adductor muscle away from the spire. In another sample of commercial shells from Cathedral Rock (data not shown), some 10 km from Hopkins I., 23% of the shells (N=140) had brown rings although they were neither drilled nor bored, indicating variability in the rate of deposition of brown rings between sites. Here, too, drilling and boring both caused significant increases in the incidence of brown rings.

Discussion

The two techniques we used to estimate growth rates, mark-recapture data and analysis of 'snapshot' length-frequency data, have generally given consistent results (Shepherd 1988, Shepherd *et al.* 1992). However, differences between them can occur due to biases associated with each (reviewed by Day and Fleming 1992). Hence the multiple growth rate values given for two sites (Table 1) could reflect possible biases. The only large discrepancy was at Ward Island, and the first estimate given in Shepherd *et al.* (1992) was probably improved by the addition of more recapture data reflected in the later estimate.

Earlier studies on the deposition of rings in abalone (Erasmus *et al.* 1994; Shepherd *et al.* 1995a,b) have shown individual variability in the rate of ring deposition, but the cause(s) have not been elucidated. Our study gives some clarification to this issue. Variability has been shown both between and within sites, and in both cases we hypothesise a dependency on the growth rate.

A comparison of sites suggests that, where the mean annual growth rate is in the range 15– 23 (–25) mm yr^{-1}, 2 rings yr^{-1} are laid down, and at sites of faster growth 3 rings yr^{-1} are laid down. The same transitional growth rate region is evident in Figure 3. The data for McLaren Point is especially interesting because it suggests that a slight reduction in growth rate compared with nearby Taylor Island, as may occur at sexual maturity, can be sufficient to change the pattern. We do not know what happens when growth rates are < 15 mm yr^{-1}, although the recapture data (Table 3) suggest that <2 rings yr^{-1} are likely to be laid down.

Within site variation also occurs and our data on rings laid down in cultured shells show that 46–72% of the variation in ring deposition is explained by differences in individual growth rate. Natural variation in growth rate in wild populations of abalone is notoriously high (Day and Fleming 1992), so this may explain much of the variability we have found. For example, the West Island sample shows the highest variability (Table 2), which may reflect the rarity of this abalone there (Shepherd and Brown 1993), and our sampling of several reefs where growth rates differed (Shepherd 1987).

However, our growth-rate dependency hypothesis does not explain why very close to an integer number of rings is apparently laid down annually by this species at every site. If the rate of deposition were wholly dependent on the growth rate we would expect continuous variation rather than a punctuated pattern. So we propose that the deposition of rings may be linked to an exogenous cue such as sea temperature and/or spawning which predisposes this species to deposit 2 or 3 rings yr^{-1} according to prevailing growth rates. Consistent with this hypothesis, ring deposition of *H. laevigata* at Taylor Island is restricted to three specific times of the year (Fig. 4), corresponding with winter and summer temperature extremes and spawning. In two other species of abalone (Shepherd *et al.* 1995b, Shepherd and Huchette 1997) rings are deposited near the time of sea temperature extremes. Erasmus *et al.* (1994) argued that ring deposition was endogenous because a constant number of rings was laid down annually in shells of *H. midae* grown in culture at a constant temperature. This is quite possible because endogenous rhythms may also be linked with exogenous cues (Tevesz and Carter 1980). If ring deposition is related to some stress, as argued by some, then the question arises whether the removal of an abalone from the water for marking might affect the pattern of ring deposition and so cause a bias. Our few data (Table 4) suggests this does not occur, although a brown ring does sometimes seem to be laid down at about the time of tagging whether it is done in or out of the water (see results).

Shell erosion and parasitism

Erosion of the shell is conspicuous in *H. laevigata* due to exposure of the nacre and occurs where the abalone lives in a habitat strongly abraded by sand, or in places where the epizoic limpet, *Patelloida nigrosulcata* is common. We have not examined critically the association between them except to observe a correlation between the presence of the limpet and shinyback shells. Wells (1988) describes a similar association between this limpet and *H. roei* in Western Australia. We tentatively suggest that the limpet by its grazing activity either directly abrades the periostracum and nacre with its radula or by removing epizoic, filamentous algae exposes the shell to erosion by sand. Both possibilities may be true. In Sceale Bay about 28% of the shells were shinybacks with epizoic limpets. Only 2 of these (c.1%) also had polychaete parasites so the presence of the limpets may reduce the incidence of parasitism in shells.

The presence of boring polychaetes in the shell and of the epizoic limpet *Patelloida nigrosulcata* was not common at our study sites except at Sceale Bay (Fig. 5). There are two possible explanations for the apparent increase in the rate of deposition of rings in parasitised shells compared with "normal" ones. The irritation may cause the abalone to deposit more rings, as shown by the tagged shells from Taylor I., or alternatively, the growth rate of parasitised shells may be lower as the animal diverts relatively more energy into shell repair than into growth, or both may be true.

Brown rings

Brown rings are much less conspicuous in *H. laevigata* than in *H. scalaris* (see discussion in Shepherd and Huchette 1997), so they can be easily overlooked. Our data show that drilling by muricid snails and boring by polychaetes stimulate the production of brown rings and that even the disturbance from tagging may apparently induce their deposition. Thomas and Day (1995) mimicked the drilling of a muricid snail in the shell of *H. rubra* and induced the deposition of a black proteinaceous layer around the hole. This is the same phenomenon.

Brown rings are rich in conchiolin and their presence as adventitious layers deposited in response to shell penetration by foreign bodies or contaminants is well known among molluscs (Tevesz and Carter 1980). Their presence in abalone shells, once recognised and allowed for, does not present a problem for aging.

Use of ring counts for aging shells

The use of ring counts for aging requires that they be laid down with consistent frequency and that biases can be eliminated (Day and Fleming 1992). Our data show that rings do not accurately tell the age of every individual but they should give an unbiased probabilistic age for a population sample. For many sites examined validation has been achieved only for the first four to six years of life during the linear phase of growth. The mark-recapture data and the data on timing of ring deposition (Fig. 4) extend the validation by implication to older age classes, but further validation is still desirable, given the variability we have encountered. It is possible, for example, that the deposition rate could change in older shells as found by Shepherd and Turrubiates (1997) in *H. fulgens*.

Our data show that biases in aging can occur through parasitism of the shell, which leads to over-estimation of age, and erosion of the shell, which leads to under-estimation. Parasitism of the shell by boring polychaetes is a more difficult problem because shells may vary from being mildly to heavily parasitised, and there is an element of subjectivity in deciding when to exclude a shell from aging. The optimal solution here is to classify shells according to the extent of parasitism as done by Shepherd and Huchette (1997) and estimate the contribution to the number of rings by the "parasitism" effect. This, of course, requires a good understanding of the growth rate, so we recommend that shell-aging should be done routinely as a part of mark-recapture studies. It does not follow from this dependency on the growth rate and the consequent variability that aging of the shell *per se* has little use. Once validation is achieved it becomes a valuable tool for deriving an age-length key and for estimating mortality which are otherwise intractable or very costly.

The bias caused by shell erosion seems not to be serious for *H. laevigata* except where "shinybacks"

are common. Our studies so far suggest that the phenomenon is restricted to shallow-water populations in the warmer parts of this species' range (unpublished data), and at a few sites where sand abrasion is common.

Acknowledgments

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Studies on southern Australian abalone (genus *Haliotis*) XVIII. Ring formation in *H. scalaris*

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Abstract

The abalone *Haliotis scalaris* deposits two fine rings a year in the spire, one in about December and the other from May to July, corresponding roughly to summer and winter sea temperature extremes. Additional fine rings, and in particular brown rings, are deposited in response to boring annelids and drilling muricid gastropods. Multiple regression analysis revealed a relationship between the number of rings in the spire and the variables: age of the shell, extent of infestation by annelids, and the number of brown rings. The analysis showed that additional fine rings and brown rings are deposited according to the degree of parasitic attack.

Keywords: *Haliotis scalaris*; rings; shell aging; parasite attack; endobionts; muricid drilling.

Introduction

The use of shell rings to age the shells of abalone is now an established technique although nearly every species so far studied appears to have unique features (Shepherd *et al.* 1995; Shepherd and Avalos-Borja 1997). In *Haliotis fulgens* and *H. corrugata*, pigmented and non-pigmented rings were observed and an understanding of the pattern of deposition of each was necessary for correct interpretation (Shepherd and Turrubiates-Morales 1997; Shepherd and Avalos-Borja 1997).

Haliotis scalaris Leach is a small, non-commercial abalone which lays down pigmented and non-pigmented rings (called brown and fine rings respectively) of prismatic structure in the spire. As the growth rate of this species is well known from earlier studies (Shepherd *et al.*1988) we examined the pattern of deposition of rings in relation to growth and intensity of infestation of the shell by parasites, in order to evaluate the use of rings to age the shell. Earlier studies (Shepherd 1973 and see review of Shepherd and Breen 1992) have shown that the main parasites of this abalone at West Island are annelids, especially *Polydora* spp., which are endobionts in the shell, and muricid gastropods (mainly *Haustrum baileyanum*) which drill holes in the shell through which they feed (Thomas and Day 1995).

Materials and methods

Monthly samples of 10–15 shells (142 in all) of *H. scalaris* in the size range 19–88 mm shell length(SL) were taken at West Island, South Australia (35°37'S; 138°35'E), from November 1993 to February 1995. The spires of the shells were ground horizontally with an electric sander until a minute hole appeared, polished with 200–600 grit fine emery paper (see Shepherd *et al.* 1995), and then etched with dilute HCl. The shell length was measured and the number of fine (i.e. non-pigmented) and brown (i.e. pigmented) rings counted. Unreadable shells (see below) were discarded.

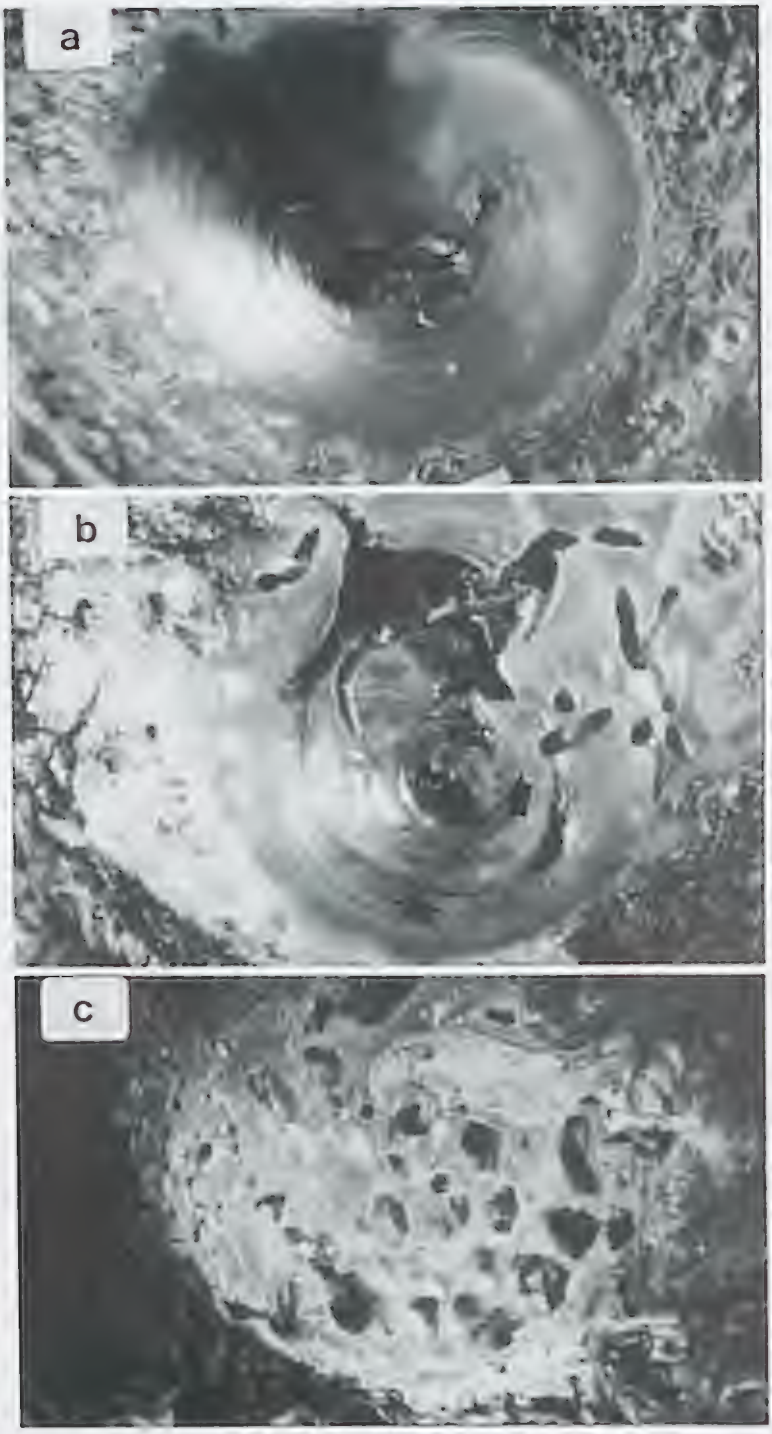

Figure 1 a – Photograph of horizontal section in the spire of *H. scalaris* (infestation score 0) showing 12 fine rings; b – Horizontal section in the spire of *H. scalaris* (infestation score 1) showing (arrowheads) brown rings; c – Horizontal section in the spire of *H. scalaris* (infestation score 3) which is unreadable.

The extent of infestation of each shell by annelid parasites in the region of the spire was estimated on an infestation scale ranging from 0 (no parasites) to 3 (heavily infested and often unreadable) and the number of muricid bore-holes counted.

Results

The spires of shells, uninfested, slightly infested and with brown rings, and heavily infested, are shown in Fig. 1. Brown rings are conspicuous because they are impregnated with pigmented conchiolin-like material, but often the polishing process leaves deep excavations in the section where the conchiolin deposits have been removed (Fig. 1 b).

The incidence of infestation is low in shells < ~50 mm SL but then increases sharply with shell size so that nearly all shells > 75 mm SL are heavily infested and unreadable (Fig. 2). In addition 9% of the shells in the range 57–75 mm SL had been drilled once or more times by muricids, probably *Haustrum baileyanum*, which is common in the vicinity. A plot of the total number of fine and brown rings vs shell length, in which we distinguish shells according to degree of infestation and presence of brown rings (Fig. 2), shows a clear tendency for seriously infested shells with brown rings to have more fine rings for a given length than shells with light or no infestation. As the growth rate of *H. scalaris* is linear with length over the length range examined (Shepherd *et al.*

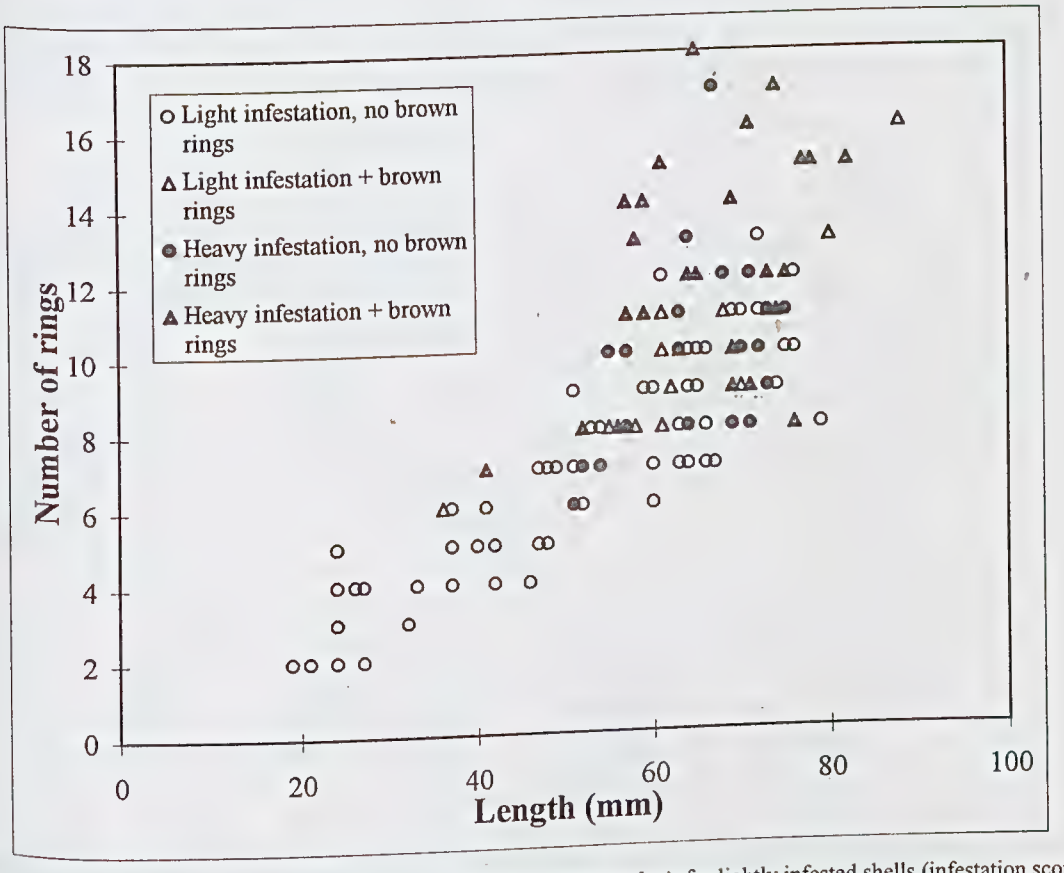

Figure 2. Plot of total number of rings vs shell length of shells of *H. scalaris* for lightly infested shells (infestation score 0 and 1) and more severely infested shells (infestation score 2 and 3) with and without brown rings.

1988), length is a good estimator of age, so shell length (SL) in mm was converted to age (A) in years by the relation:

$$SL = 0.008 + 13.61\ A$$

This equation indicates a mean growth rate of 13.6 mm a year (Shepherd *et al.* 1988). A regression of the number of rings (R) vs age of the subset of shells with an infestation score of zero and without brown rings gave the regression equation:

$$R = -0.53 + 1.96\ A\ (R^2=0.77;\ N=59)$$

The standard error of the slope was 0.13 indicating that the slope did not differ significantly from the integer 2. So we concluded that 2 fine rings a year were laid down in these shells.

Effect of infestation and muricid bore-holes

Of the 11 shells with bore-holes in them, there was a significant correlation between the number of brown rings and the number of bore-holes ($r=0.63$; $P<0.05$). Two of these shells had bore-holes but no brown rings. In addition, there were 29 other shells with brown rings and a mean infestation score of 2.0 (s.e.0.1) but no detectable bore-holes. A multiple linear regression analysis of the total number of fine *and* brown rings (R) vs age (A), the number of brown rings (BR), and the infestation score (I), after excluding unreadable shells, gave the regression equation (standard errors in brackets):

$$R = -0.56(0.71) + 1.93(0.17)\ A + 1.05(0.22)\ BR + 0.48(0.22)I\ (R^2=0.70;\ N=118)$$

The constant did not differ significantly from zero ($t=0.8$; ns), but the other coefficients were significant (for A, $t=11.4$, $P<0.0001$; for BR $t=4.8$, $P<0.0001$; for I $t=2.2$, $P<0.05$). Thus, for all readable shells age accounted for 2 rings a year, boring of the shell for 0.5 rings per unit infestation score, and brown rings for one ring. This last constant means that brown rings are themselves adventitious and do not cause the deposition of extra rings.

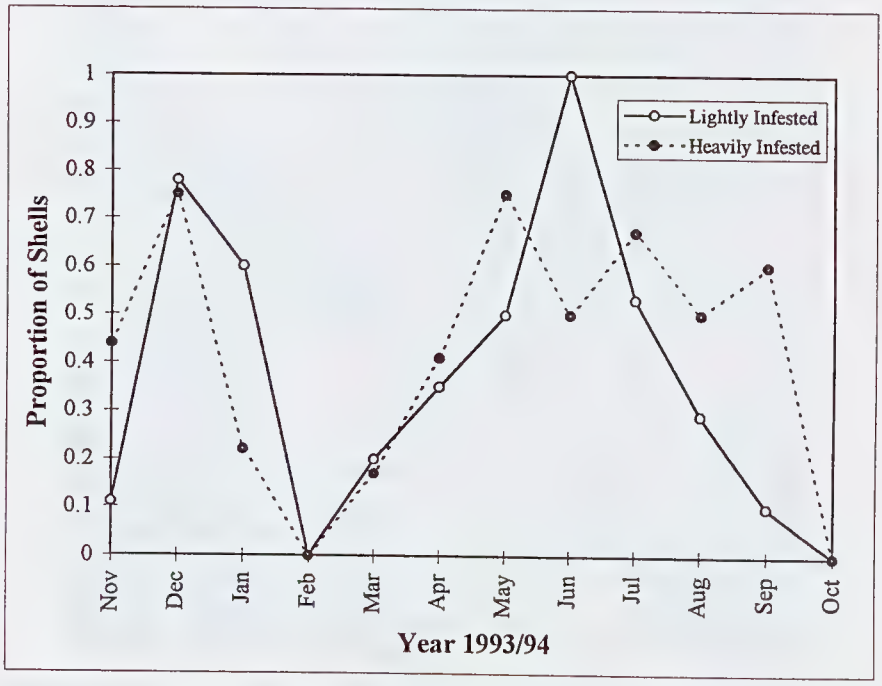

Figure 3. Monthly distribution of proportion of shells of *H. scalaris* with a recently deposited ring in lightly infested shells (infestation score 0 and 1) shown in open circles,and more seriously infested shells (infestation score 2 and 3) shown in closed circles.

Timing of ring deposition

When viewed in horizontal section prismatic rings are opaque whereas layers of aragonite are translucent. Thus, examination of the interior surface of the shell near the spire in monthly samples indicates the approximate months in which rings were deposited. This was confirmed where possible by examination of the innermost ring in the horizontal section at the spire. A plot of the percentage frequency of recently deposited rings by month for (a) shells with infestation scores of 0 or 1, and (b) shells with infestation scores of 2 or 3 (Fig. 3) shows that ring deposition is concentrated in the May to July period and December in the slightly infested group of shells but more diffusely around the same seasonal peaks in the more seriously infested group of shells.

Discussion

The pattern of deposition of rings in *H. scalaris* differs from that of other abalone species which lay down one ring a year (Prince *et al.* 1988; Erasmus *et al.* 1994; Shepherd and Avalos-Borja 1997) or a variable number of rings (Shepherd and Turrubiates 1997). In *H. scalaris* ring deposition occurs as sea temperatures approach their respective maxima and minima (see Shepherd and Womersley 1970). Possibly sea temperature is the exogenous factor controlling ring deposition in this species (reviewed by Tevesz and Carter 1980 for bivalve molluscs) although seasonal variation in day length would equally well explain our data.

Infested shells clearly have more fine rings for a given size than uninfested shells (Fig. 2). There are two possible causes of this. One is that infested shells grow more slowly as the infested abalone devotes relatively more energy to shell repair than to growth (Shepherd and Breen 1992). Hence infested shells would be smaller for a given age than uninfested shells and so would appear to have deposited more than two rings a year. Alternatively, extra fine rings may be deposited in response to infestation. Gabriel (1981) found that calcite was more resistant to borers than aragonite, so deposition of prismatic layers would be adaptive. Our data are consistent with both hypotheses, but the large number of extra fine rings laid down in infested shells suggest that the latter effect (extra rings) is the greater of the two. Shepherd and Triantafillos (1997) also concluded that polydorid boring induced extra rings to be deposited in *H. laevigata*. The correlation between the number of muricid bore-holes and the number of brown rings and the multiple regression analysis together support the hypothesis that drilling by muricid snails causes the deposition of brown rings. Thomas and Day (1995) found that artificial holes which mimicked the drilling of muricids caused *H. rubra* to secrete a black proteinaceous deposit around the holes, and our own observations on many shells of *H. scalaris* confirm that brown deposits around bore-holes is a common response to drilling by *H. baileyanum*. However, the occurrence of brown layers (or rings) in the spire of infested shells without bore-holes suggests that deposition of brown rings is a general response to irritation by parasites or endobionts of the shell, and that the brown ring is not restricted to the precise location of the parasite's attack.

The relation between the brown rings observed by us in *H. scalaris* and *H. laevigata* (Shepherd and Triantafillos 1997) and the brown ring disease widely reported in bivalves (Paillard *et al.* 1994; Perkins 1996) is unclear. Both are characterised by brown conchiolin deposits on the inner surface of the shell and are associated with irritation of the mantle by parasites and commensals. However, in the case of the brown ring disease the causative agent is a species of *Vibrio* which can lead to high mortalities, whereas in abalone there is no evidence that the brown rings are pathological.

How useful are rings for aging *H. scalaris* given the several apparent causes of ring deposition? Discarding a large proportion of a sample because of shell infestation, as Nash (1992) found necessary for *H. rubra*, could seriously bias estimates of the mortality rate obtained by catch-curve analysis, given that infestation increases with age (Shepherd 1973; Clavier 1992), and the oldest age classes strongly influence the analysis. An advantage of this multiple regression technique is that fewer shells need to be discarded (in this study 20% instead of over 50% which were infested) so the bias in the resultant estimate should be much reduced. The other advantage of the technique is the

disclosure of secondary causes of ring deposition, and the determination of the strength of their effect. The disadvantage is that, because these secondary causes of ring deposition as well as the growth rate are likely to vary from place to place, growth studies will need to be carried out at practically every site where shell aging is to be applied. This reduces but does not nullify the usefulness of the technique.

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Variability in larval settlement of abalone on artificial collectors

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Abstract

The spatial and temporal variability in settlement of greenlip abalone, *H. laevigata* on artificial settlement collectors was examined over three years. Pairs of the collectors of an improved design were deployed on the sea bed at six sites over a number of different spatial scales. A period of peak settlement occurred between October and December in each of the three years at all sites. The similarity in size frequencies between sites at the settlement peaks demonstrated that spawning was epidemic, occurring over a period of 2–3 days. The synchronous timing and intensity of peaks at four sites within 2 km of each other contrasted with that of a site 8 km distant. Adult densities between sites during the spawning season ranged from 0.0 m^{-2} to 1.2 m^{-2}. Over this range of densities, numbers of larval settlers were independent of local adult abundance.

Collectors are a useful tool in examining inter-annual variability in the timing and synchrony of spawning and in measuring the relative abundance of abalone larvae. Growth rates inferred from "cohorts" followed at two week intervals allowed settlement/spawning times to be more accurately estimated than with previous methods such as gonad indices.

Keywords: *H. laevigata*, larval settlement, larval collectors, spawning, epidemic spawning.

Introduction

Artificial collectors have been successful in measuring the intensity of larval settlement in many marine groups, including lobster (Booth and Tarring 1986, Phillips 1986), echinoderms (Harrold *et al.* 1991, Keesing *et al.* 1993), scallops (Sause *et al.* 1987) and abalone (Keesing *et al.* 1995, Nash *et al.* 1995). Estimation of the relative abundance of larvae on both a temporal and spatial scale is important for determining inter-annual variation of settlement and to establish the scale of larval dispersal in order to begin to understand metapopulation structures in the area.

Keesing *et al.* (1995) used a modified abalone larval collector based on that used by Nash *et al.* (1995) and highlighted several problems associated with the laserlight plate collector design. The high sediment and degree of algal fouling after four weeks made sorting the samples time consuming, the surface area of the collector was simple and limited and the collector had minimal trapping capabilities. The development and field use of a novel collector design, incorporating a variety of surface orientations and light regimes that may be important to settling larvae, is described in this paper. The collector must be able to capture enough abalone larvae to give enough power to detect any patterns in settlement. Using the new design, questions relating to temporal and spatial variability in larval settlement can be addressed.

South Australia has five species of abalone, *Haliotis laevigata* Donovan, *H. rubra* Leach, *H. cyclobates* Peron and Lesueur, *H. roei* Gray and *H. scalaris* Leach. Only the first two are commercially fished. This study set out to determine the temporal and spatial variability in larval

greenlip abalone (*H. laevigata)* settlement at six sites. We compared the larval abundance at sites of high and low adult abundance on Taylor Island and McLaren Point. Temporal variation was measured by comparing larval settlement at several sites over a three year period. By deploying collectors in the field from August to February some years, we were also able to determine blacklip (*H. rubra*) settlement.

Materials and Methods

Collectors

At the original site used by Keesing *et al.* 1995 (McLaren Point, South Australia), we compared the larval collecting ability of three artificial collector designs, including the original laserlight plate design (type 1– see Keesing *et al.* 1995). Collector type 2 was constructed of 13 layers of a black polycarbonate material which had a complex surface area (3 m^2) and a volume of 0.3m^3 (Fig.1). The surface of neighbouring sheets formed channels which provided a variety of surface orientations and an enhanced trapping capability. It also provided a variety of light regimes to incorporate any larval preference. Type 3 was constructed of 10 layers of fluorescent diffuser material held separate with a central rod which directed the collector into the current.

Study sites

Once comparisons were made between collectors, the most efficient collector design was chosen and used to establish spatial variation in larval settlement at five sites in Thorny Passage, near Port Lincoln. In addition, the relationship between adult abundance and the number of larval abalone

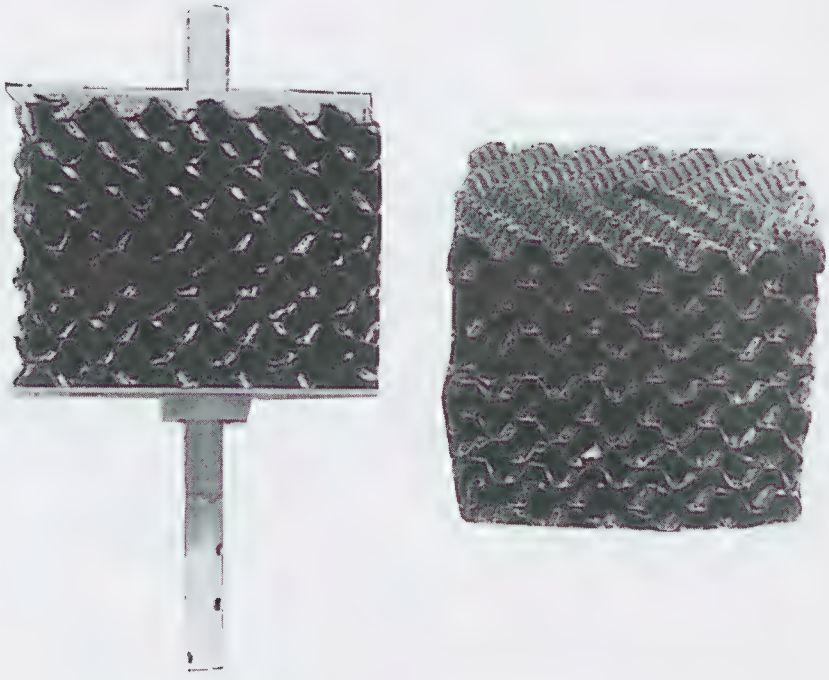


Figure 1. Collector design 2 used in this study.

settling was compared by choosing sites of varying adult abundance. Four of the sites (two high and two low adult abalone abundance) were located 2 km apart on Taylor Island (136°1'00", 34°54'00"). An additional site (Passage site) with no known adult population of *H. laevigata* within 500m (P. Clarkson pers. comm.) was set 2 km west of Taylor Island. The remaining site (McLaren Point – 136°1'00", 34°47'90") with a high adult abundance, was 8 km north of Taylor Island (Fig.2).

At each site, six collectors were placed one metre above the seabed. The collectors were fixed in pairs to a solid yoke attached to a cement-filled tyre. During the experimental period we changed the design of the yoke from PVC piping to a flat mild steel. This was especially useful in areas of high water movement where the PVC piping was prone to break.

The distance between pairs of collectors varied from 2–6 m. The collectors were placed at a water depth of 5–6 m at four sites and at 11–12 m at the remaining two sites. Five of the sites were on the sand-reef interface near an adult greenlip abalone population and the remaining site (Passage site) was on a sandy bottom.

The collectors were conditioned naturally in the sea with diatoms and bacteria which created a

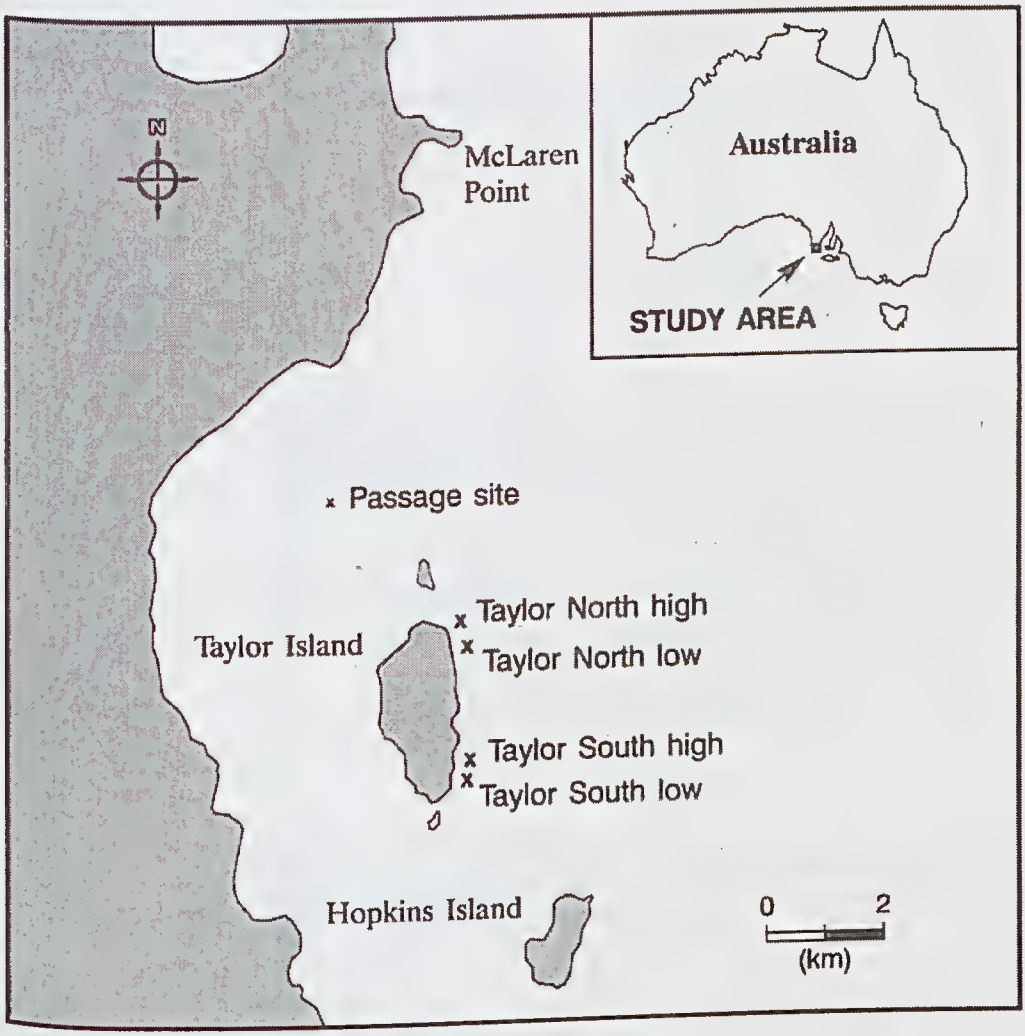


Figure 2. Study site locations in Thorny Passage, near Port Lincoln, S.A.

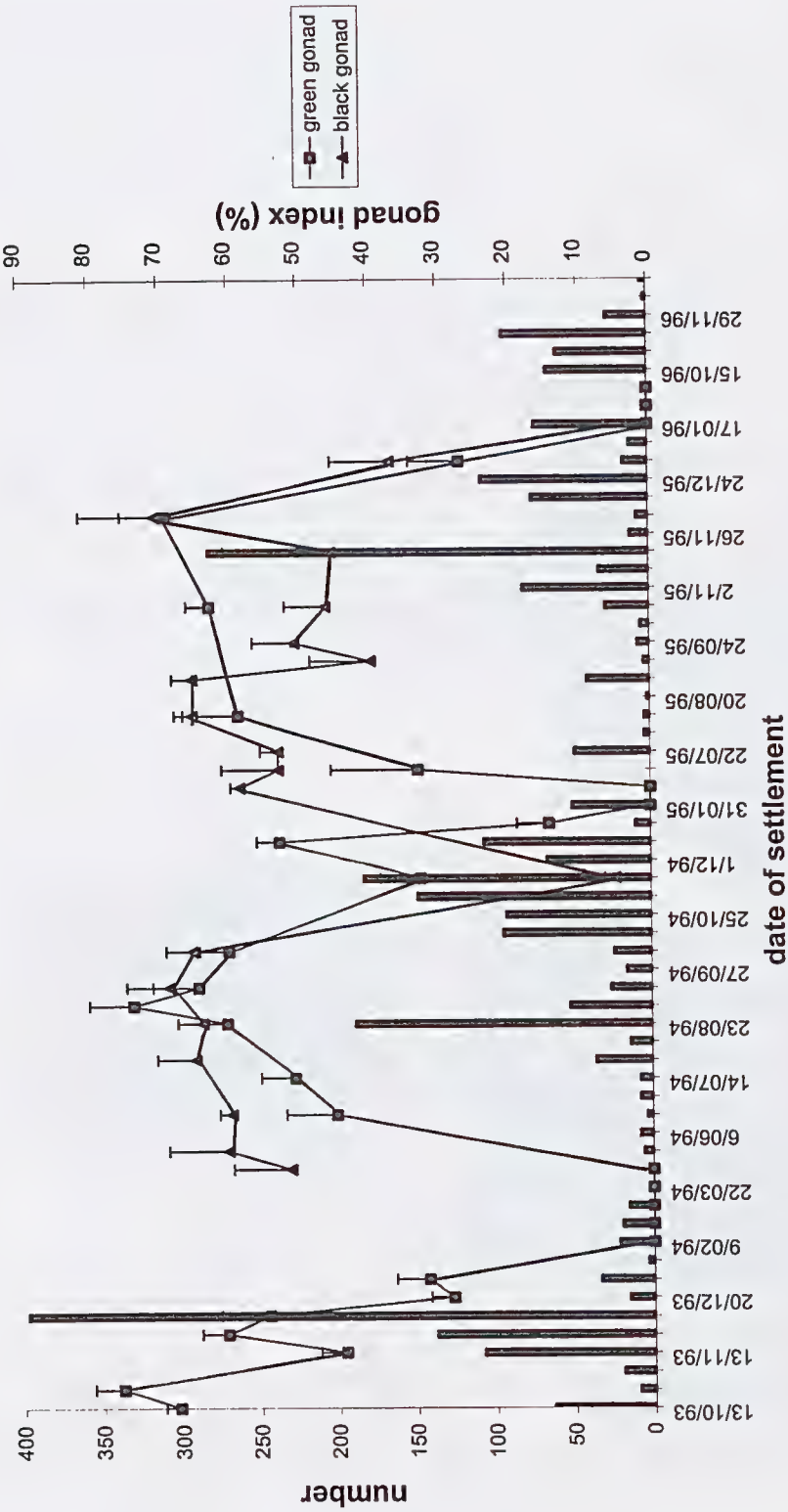


Figure 3. Total number of post-larvae in the collectors at the Taylor South high site from 1993–96 and the mean gonad indices for greenlip (*H. laevigata*) and blacklip (*H.rubra*) at the same site over the same period. Vertical bars are the standard errors.

suitable substratum for settlement and growth of juvenile abalone (Nash *et al.* 1995, R.Grove- Jones pers. comm.).

Collectors were monitored for five months over the summer spawning season at five sites and all year round at one site (Taylor South high). Deployment times of between two and four weeks were used according to Keesing *et al.* (1995). Two replicate pairs of collectors were picked up every fortnight, each pair representing two and four week collections. The collectors were brought to the surface and placed into tagged bags on the boat. All retrieved collectors were replaced with new collectors. Hence at all times there were two conditioned collectors *in situ* to allow larval settlement to occur. The retrieved collectors were returned to the laboratory and frozen until examination. The collectors were then thawed and washed thoroughly with fresh water to remove any settled abalone.

Scrubbing of the collectors to remove juvenile abalone was unnecessary. In a pilot experiment to determine the benefit of scrubbing the collectors we found that we retrieved only 1.4 % (SE = 0.74, n=6) more abalone. All sediment greater than 125μm was collected and stored in 100% ethanol. Small amounts of the dye Rose Bengal were added to distinguish animal tissue from sand to make sorting easier. Each sample was later examined under a dissecting microscope and the number and size of abalone found were recorded.

Species identification was difficult under a binocular microscope if the post-settlement abalone were less than 500μm shell length (SL). Abalone larger than this (as found on the four week collectors) were able to be identified by comparing shell shape with blacklip and greenlip abalone reared in the laboratory. In order to link the settlement peak with a specific species, we examined all four week collectors around the settlement peak. In addition, gonad indices taken year round gave us some idea of spawning activity among the different species.

Growth rates of juvenile abalone

The growth rate of abalone on the collectors was determined by following the prominent cohorts from two collectors, placed on the seabed on the same date but retrieved two and four weeks later, respectively. A linear growth model was assumed during the first four weeks.

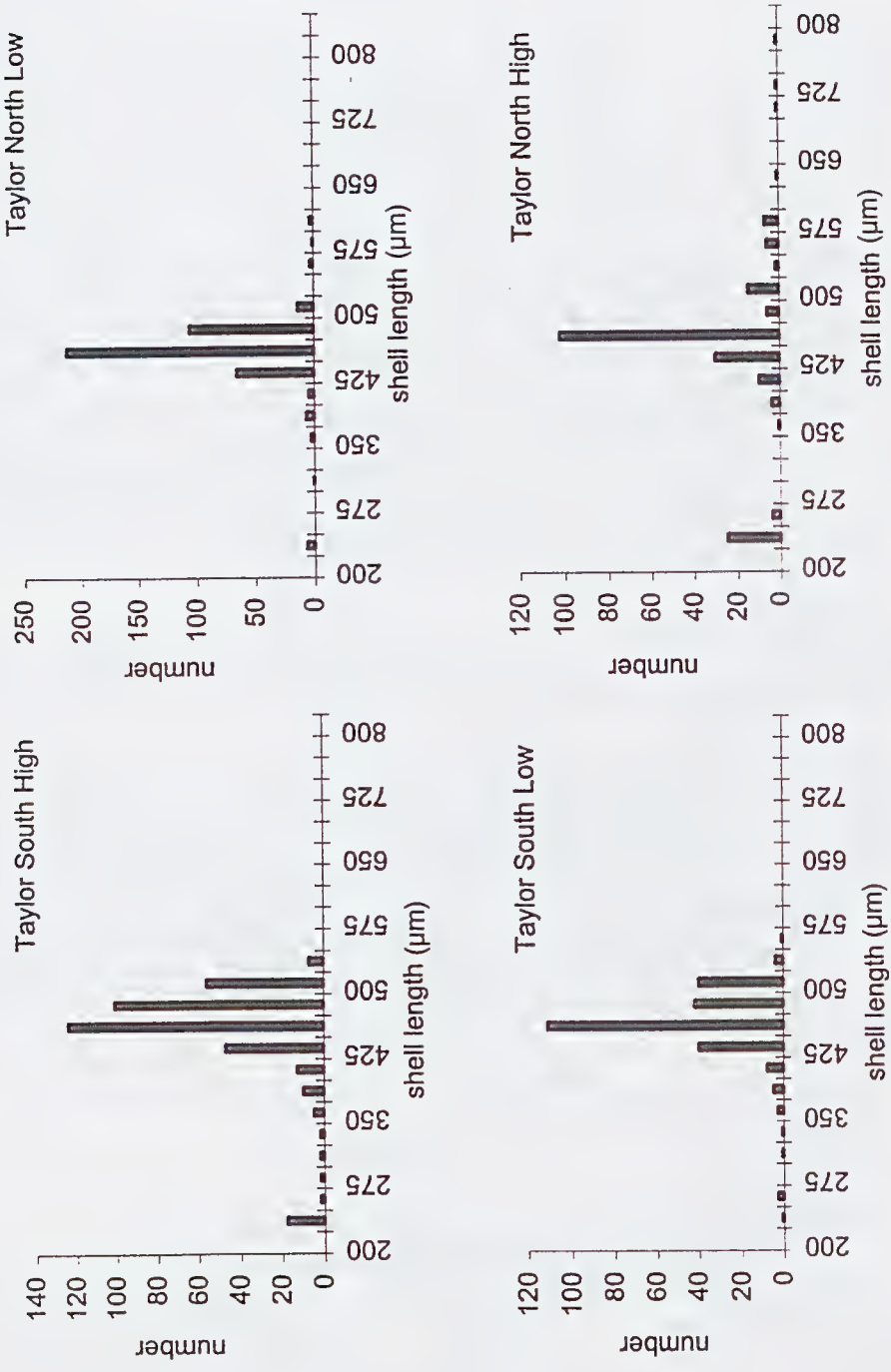
Gonad Indices

Once a month, at the time of retrieval of a collector, 6–10 samples of gonad from some or all of the three most commonly found species at the Taylor South high site (*H. laevigata*, *H. rubra* and *H. cyclobates*) were taken to indicate reproductive condition and hence spawning activity. All individuals were of reproductive age (over 120 mm SL for *H. laevigata* and *H. rubra* and over 40 mm SL for *H. cyclobates*). Samples were stored frozen or fixed in 10% formalin for later examination. Gonad indices were measured by slicing a cross section through the visceral mass at a point midway between the spiral coil and tip. The cross section was then traced onto paper and enlarged 5 times by photocopier and weighed. The gonad portion was weighed relative to the visceral mass (to the nearest 10 mg) and the gonad index determined by the following equation (after Shepherd and Laws 1974):

$$\text{Gonad Index} = \frac{\text{weight of gonad}}{\text{weight of gut + gonad}} \times 100$$

Adult densities

At each of the six sites, two permanent 50 m long transects were marked. Annual summer surveys one metre either side of the transect line recorded the number and size frequency of all greenlip abalone found. From the size frequency data, it was possible to distinguish cohorts relating to distinct age classes to at least four years old (Shepherd *et al.* 1992). The relationship between the density of reproductively active abalone (> 100mm SL) and the density of larvae at the peak settlement on the collectors at each site was tested by ANOVA. The density of adults was assumed to be relatively stable in the area between the time of peak settlement and survey of adults (conducted in November each year).


Taylor North Low
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Taylor South High
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number
shell length (µm)
0
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425
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650
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800

Results

Collector type

We examined qualitatively the relative time spent cleaning the collector after retrieval and time spent sorting samples as important factors in choosing a practical design. Unpublished results by the authors show that, comparatively, the black multilayered polycarbonate collector was equally efficient at capturing abalone larvae as the type 1 collector and superior to type 3. The accumulation of algal growth on collector types 1 and 3 over a short exposure time of two weeks increased sorting time. The black polycarbonate sheets (type 2) retained very little algae and therefore reduced sorting time. In addition, the complex surface area of type 2 also provided a variety of light and surface orientations to accommodate any larval preferences. Type 2, the black polycarbonate collector, was therefore chosen as the optimal design for our purposes. At each collector pickup, two replicate samples were taken for each period of deployment (two and four weeks). A linear regression analysis demonstrated no significant difference in the number of abalone found between replicate collectors ($F=251.5$ $r^2=0.79$, $n=70$), thus justifying the utility of the collectors as a quantitative tool.

Larval settlement

The numbers of settlers recovered from the collectors (Fig. 3) and their size composition (Fig.4) indicate that, while there is a low continuous level of settlement throughout the year, short intense periods of settlement occurred at each site. Data are shown for the Taylor South high site where the data set is most complete (Fig. 3). Because the replicates were not significantly different, the data represent the sum of abalone found on the two collectors. At this site collectors were deployed year round in 1994/1995 and over the spring-summer period for the other three years. The peaks are likely to represent a *H. rubra* (August) and a *H. laevigata* (October–December) settlement, as suggested by the decline in the respective gonad indices of the two species (Fig. 3).

The peak *H. laevigata* settlement in 1993 was synchronous at the four Taylor Island sites, and asynchronous with the peak settlement at the site 8 km distant (McLaren Point) (Fig. 5). This spatial asynchrony was repeated in the subsequent years of study (Fig. 6). The first year's data showed peaks of similar intensity at all sites. Although settlement was markedly less at three of the Taylor Island sites in the last two years of study, the peak was still synchronous at all Taylor Island sites, including the Passage site.

The timing of peak settlement of *H. laevigata* at the Taylor Island sites varied over the three year study but always occurred during the late spring or early summer (early December 1993, late October 1994 and mid December 1995).

The modal mean size of post-larvae was 250μm SL on the 2-week collector retrieved on 12 Nov. 1993 and 475μm SL on the 4-week collector retrieved 2 weeks later. This gave a mean growth rate of

Table 1. The relationship between densities (per meter square) of reproductive adult *H. laevigata* and post-larvae on the collectors. Larval density was taken from the peak settlement occurring over summer.

		Taylor South high	Taylor South low	Taylor North low	Taylor North high	Passage	McLaren Point
1993	Adults	1.15	0.37	0.49	0.75	–	0.64
	post-larvae	65.0	43.3	70.0	37.7	–	56.0
1994	Adults	0.51	0.28	0.42	0.66	0.0	0.45
	post-larvae	66.0	–	–	35.0	10.7	34.7
1995	Adults	0.49	–	0.25	0.42	0.0	0.45
	post-larvae	57.0	–	16.7	11.3	35.6	52.7

– indicates that no data was taken

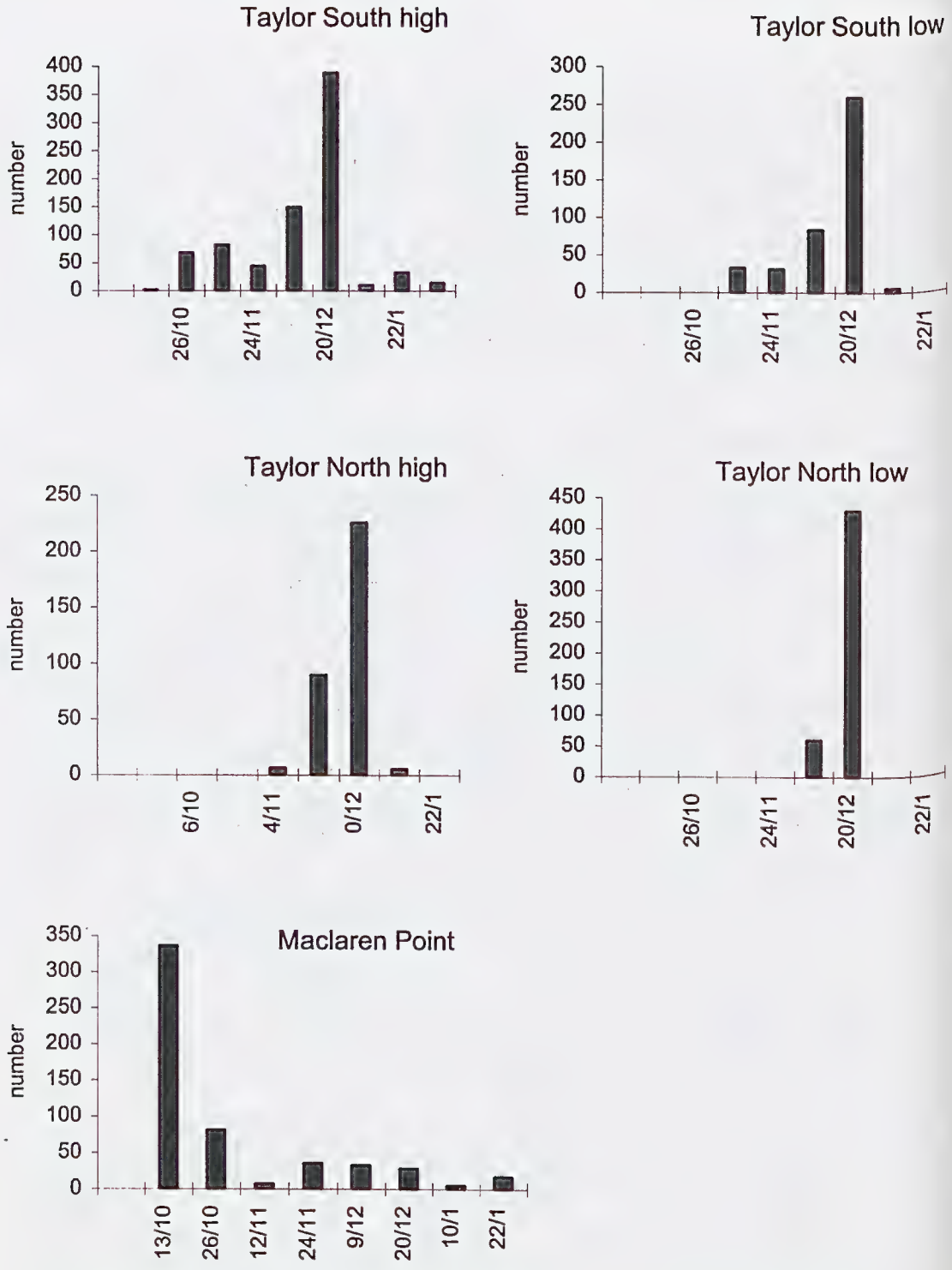

Figure 5. Total number of abalone found on the collectors at all sites in the period October 1993–January 1994.

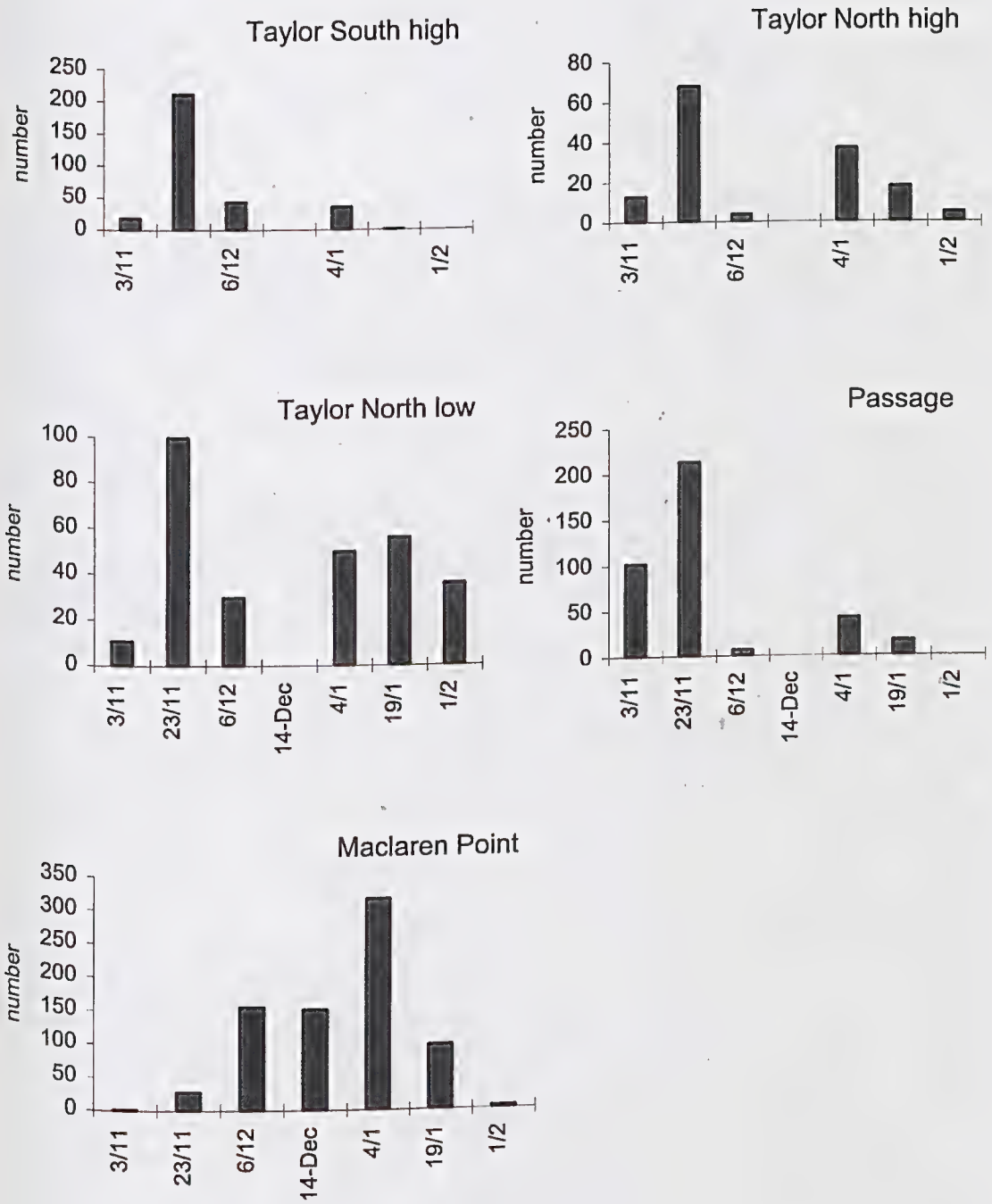

Figure 6. Total number of abalone found on the collectors at all sites in the period November 1995–February 1996.

19μm day^{-1}. Comparable growth rates were found by comparing the size frequency of abalone on several other 2 and 4-week collectors.

Annual surveys over three years produced mean adult densities of 0.66 m^{-2} (SE 0.11, n=6) and 0.36 m^{-2} (SE 0.16, n=5) at the high and low density sites, respectively. At McLaren Point, adult density was 0.51 m^{-2} (SE 0.06, n=3), and at the Passage site, adult density was zero (Table 1). The density of settled larvae at peak settlement ranged between 10.7 and 70.0 m^{-2} and was not related to the density of reproductive greenlip abalone (ANOVA : $F=5.31$, $P<0.001$).

Discussion

The synchronous timing and intensity of the peaks of settlement at the Taylor Island sites within 2 km of each other contrasted with those found at a site 8 km distant. Although our samples represent the cumulative number of surviving settlers over two weeks, the similar size composition of settled abalone collected during the peak of settlement activity (Fig. 4) is indicative of an intense period of settlement perhaps lasting just 2–3 days and suggestive of a epidemic spawning some days before. The low or background density of settlement before and after the peak may represent widely dispersed settlers from a more distant locality or settlers from a local asynchronously spawning parent population.

Were the larvae captured by the collectors conspecific? The reef habitat along Taylor Island supports low to medium density populations of *H. scalaris* and *H. rubra*, and *H. laevigata* can be found at high (Taylor South high, Taylor North high) and low (Taylor South low and Taylor North low) abundances. *H. cyclobates* is common at two sites (Taylor South high, Passage site) while *H. roei* is uncommon at all but one site (McLaren Point). To derive which abalone species were likley to be spawning, we examined gonad indices of the more abundant species present at Taylor South high. With the exception of the 1996/1997 season, the reduction in gonad indices, corresponding with spawning, suggests that spawning episodes of the 4 species occur at different times of the year. *H. scalaris'* peak reproductive activity occurs in March– April, and *H. cyclobates* spawned during winter (1994) and during early to late summer in 1993/1994 and 1995/1996 (unpublished data). *H. roei* is in reproductive condition all year round, but has a major spawning season in April (Keesing *et al.* 1995) and the larvae settle in shallow reef habitat (1–2 m deep) (Shepherd and Laws 1974). It is therefore unlikely that larval settlement of these three species would be recorded on the collectors during the experimental period, or be confused with *H. laevigata* or *H. rubra* settlement peaks.

The differences in settlement recorded between sites 8 km distant may be due to differences in timing of spawning at the two sites. Water temperatures and food availability differ substantially between the two sites and may induce different spawning periodicities. Water temperature and weather variables at the sites are being assessed as environmental cues for spawning in abalone (Rodda and Keesing, in prep.).

The timing of peak settlement in *H. laevigata* at the Taylor Island sites varied slightly over the three year study although it always occurred in late spring or early summer (early December 1993, late October 1994 and mid December 1995). Similarly, at the same site in 1987, Shepherd *et al.* (1992) found that spawning took place in early December. The reproductive condition of the adults is influenced by the available nutrition during the months preceding spawning. The gonad index in *H. laevigata* begins to increase to around 50% by July each year (see Fig. 3), some 4–5 months prior to spawning, peaks at around 75% prior to spawning and tends to fall abruptly after peak settlement.

Some declines in gonad indices were not reflected in settlement peaks. Explanation of such incongruities include: the small gonad sample sizes, which may not accurately reflect the natural range of reproductive activity occurring at the sites, the resorption of gonad material by individuals or a light settlement on the collectors. Changes in periodic gonad indices, as described in this paper, derived from necessarily small sample sizes, are a valuable guide to spawning activity, but are not definitive. At our study sites, for example, they indicated likely differential settlement peaks of *H. rubra* and *H. laevigata* and also excluded the likelihood of larvae of *H. cyclobates* being confounded

with those of *H. laevigata*; *H. cyclobates* spawned 2 months after *H. laevigata* in the 1993/94 season and 2 months before it two years later (Rodda, unpublished data).

The growth rates of about 19μm per day found in this study are similar to that found by Preece *et al.* (1997) for *H. laevigata* in the first month of benthic existence. Because of the short larval period, the growth rates measured on the collectors allow us to backcalculate the date of settlement and spawning more accurately than any other way. Other studies backcalculate settlement from much older juveniles (up to 3 months old) (Sainsbury 1982, Prince *et al.* 1988, Shepherd and Daume 1996), a much cruder method due to the variability in post-larval growth. The ability to backcalculate the probable spawning date is invaluable in understanding the cues that induce spawning in *H. laevigata* (Rodda and Keesing in prep.).

There was no relationship between adult abundance and the number of abalone which settled on the collectors over a 2 km range. The Passage site, located 2 km from Taylor Island, had no adult *H. laevigata* within at least 500m, yet still produced a synchronous settlement peak with the remaining Taylor sites. The population of *H. cyclobates* in the immediate area of the Passage site is an unlikely source of settlers on the collectors, because gonad indices suggest that its spawning activity is in late summer. Shepherd *et al.* (1992) concluded that dispersal along Taylor Island was at least 200m, but our study suggests that the dispersion may be greater. Given tidal currents of 50 cm sec^{-1} or more in Thorny Passage and tidal excursions of several kilometres, larvae could be dispersed on the scale of kilometres. The synchronous peak on the Passage collectors with that of sites on Taylor Island is consistent with this view.

Artificial collectors provide a valuable tool for the study of the early life history of abalone, and associated questions such as spawning cues of adults. Our new design of collector with high capture ability allows comparisons within and between sites over time. Now the way is open to monitor larval availability to abalone habitat, and to answer the more difficult questions posed by Day and Shepherd (1995) namely, the relation between larval availability and recruitment and the factors that influence recruitment success.

Acknowledgments

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Abalone stock enhancement by larval seeding : effect of larval density on settlement and survival

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Abstract

Abalone larvae of *Haliotis rubra* and *Haliotis laevigata* were released experimentally at sites in South Australia to determine the effect of density of larval release on subsequent survival. In one experiment, densities of post-larvae of *Haliotis rubra*, 19 days after release, were highest at intermediate release densities (16,000 larvae m^{-2}) compared with low (1,600 m^{-2}) and high (80,000 m^{-2}) release densities. Highest post-larval densities were about 4.5 times background densities at control sites. In a second experiment, densities of post-larvae of *Haliotis laevigata*, 6 days after release, were three times higher (317 m^{-2}) at high release densities of 120,000 larvae m^{-2} than at low release densities of 2,000 m^{-2}. After 49 days average survival of post-larvae across treatments was about 0.5% and mean density was 3.8 m^{-2}. After 11 months the density of seed was about 0.6 m^{-2}. In both experiments the most cost-efficient densities of larval release were the lowest in terms of the proportions of released larvae settling and surviving. Given the likely density-dependent mortality of post-larvae after settlement, larval release at even lower densities than those tried over larger areas is likely to be the optimal seeding strategy.

Keywords: abalone, *Haliotis rubra*, *Haliotis laevigata*, reseeding, seeding, larvae, early juvenile, density-dependent mortality, survival, stock enhancement.

Introduction

The decline of abalone fisheries in many parts of the world has stimulated interest in the enhancement of abalone stocks. One method of enhancement, seeding with juveniles, has been practised for decades in Japan to prop up its ailing abalone fisheries (reviewed by Saito 1984, Uki 1989) and has been tested elsewhere (Schiel 1993, Tegner and Butler 1985, reviewed by McCormick *et al.* 1994). The high mortality or excessive cost of juvenile seeding has attracted sporadic interest in larval seeding. In the 1960s Ortiz-Quintanilla (1980) developed a program called "mareas de cría" for seeding abalone reefs in Baja California with larvae, and larval seeding is still practised by some co-operatives there (reviewed by Mazón-Sastegui *et al.* 1996). Larval seeding trials in Japan are referred to by Salas-Garza and Searcy-Bernal (1990). Tegner *et al.* (1986) attempted larval seeding in California by transplanting larvae into the field on mesh screens, a technique developed by the oyster industry (Jones and Jones 1983). Later Tong *et al.* (1987) and Schiel (1992) did similar small scale experiments in New Zealand with variable results.

In this paper we describe the experimental larval seeding of two commercial abalone species, *Haliotis laevigata* Donovan (greenlip) and *Haliotis rubra* Leach (blacklip) at two sites in South Australia. We first describe our technique for transporting larvae to the field site and then delivering known larval densities onto the bottom. We then describe preliminary experiments testing (a) the effectiveness of tents to retain larvae at the site (see below), and (b) different larval densities. Our purpose was to test the delivery of different densities of larvae in suitable habitat in order to determine the range of densities which produced the optimum survival 20–50 days later. An

understanding of the process of settlement and survival at a fine scale during this initial phase of the life history is a necessary prelude to seeding trials at commercially realistic scales.

Materials and methods

Site description

Two seeding sites, one for *H. rubra* and one for *H. laevigata,* were selected within 30 km of abalone hatcheries near Port Lincoln, South Australia to minimise larval transport time. The sites were chosen for the abundance of boulders of manageable size(about 10x15 cm diameter) for sampling post-larval abalone, with adequate cover of crustose coralline algae (CCA), and were at the transition from the sloping rocky shoreline to a sandy bottom, some 5–15 m offshore. Control sites in a similar habitat, two for the blacklip experiment and one for the greenlip experiment, were established within 150 m of the experimental sites. The blacklip site near Cathedral Rock (Lat. 34°59'42"S; Long. 135°59'42"E) was 7–9 m deep. *Cystophora spp.* and *Ecklonia radiata* were the dominants on granite blocks and an algal turf of filamentous species on boulders. The greenlip site on the western side of Taylor Island (Lat. 34°52'48"S; Long.135°59'54"E) was 9–11 m deep, and the dominant algae were *Ecklonia radiata, Seirococcus axillaris* and *Sargassum spinuligerum.* Under the boulders at both sites was a diverse fauna of chitons which graze the CCA assemblage and maintain a suitable substratum for settling abalone larvae (Kangas and Shepherd 1984, Clarkson and Shepherd 1985, Shepherd and Daume 1996).

Transport and release of larvae

Larvae were collected from the hatchery when they were competent to settle i.e. at stage 39 when the 3rd tubules on the cephalic tentacles appear (Hahn 1989, p.82). The larvae were placed onto damp 100 µm mesh screens and transported in insulated foam containers at about 14°C (Jones and Jones 1983). At the release site the larvae were resuspended in seawater in four 60 l barrels at the desired densities. The barrels were then sealed and the seawater was aerated by forcing air through weighted airstones connected to a SCUBA bottle. Just before release, the barrels were pressurised to about 28 kPa with a SCUBA tank. A 50 m plastic hose of 13 mm diameter was connected to the barrel at one end and fitted with a tap at the other end, so that the diver could control the release density of larvae by timing the flow of seawater and suspended larvae from the tap *in situ.* The diver released the larvae close to the bottom according to the experimental protocol at the pre-marked sites.

Seeding experiment

To ensure that our experiments tested a range of release densities, one treatment was to use tent-like enclosures designed to retain larvae injected under their canopy. For this treatment pyramid-shaped tents made of 125 µm nylon mesh, with a basal area of 1 m^2 were placed on boulder substrata. The basal perimeter was weighted with chain and the apex was kept elevated with a buoy. The tents were removed after one day at the greenlip site but after 7 days at the blacklip site due to rough seas.

We planned to test a range of larval densities over two orders of magnitude. As our pre-release estimates of density were necessarily crude, we took samples (N=5–8) either at the point of release *in situ* or from the barrels, counted the larvae in them, and so derived mean release density values. Mean densities of larvae of *H. rubra* released were: 1600, 16000 and 80000 m^{-2}, and those of *H. laevigata* were: 2000 and 120000 m^{-2}. The mean coefficient of variation was 7% for the density estimates in the *H. rubra* experiment and 11.8% in the *H. laevigata* experiment. For each density treatment there was a "tent" and "no-tent" treatment. Each treatment was carried out in four replicate 1 m^2 plots for *H. rubra* and 8 replicate 1 m^2 plots for *H. laevigata.* Each plot was sampled twice in different parts (the micro-site effect). There were twice as many replicate plots for the *H. laevigata* experiment because samples were taken from 4 plots at the first sampling and from the other 4 plots at the second sampling (see below). In all, the experimental area covered 24 m^2 for *H. rubra* and 32 m^2 for *H. laevigata.*

Each sample consisted of a set of 5–6 boulders with a mean surface area per boulder of 300 cm^2. Each sample set of boulders was sealed in a numbered plastic bag *in situ*, transported to the laboratory, and frozen for later sorting. Subsequently, the boulders were defrosted, rinsed thoroughly with freshwater, and the washings sieved through a nest of sieves. The residues retained in the sieves were stained with Rose Bengal, fixed in alcohol, and sorted under a binocular microscope. Post-larval abalone shells were counted and their shell lengths measured to the nearest 25 µm.

The *H. rubra* larvae were released at the blacklip site on 20 August 1994, nine days after spawning, and the inoculated sites were sampled 19 days later. The two control sites for this experiment were each sampled twice, one week before and one week after the sampling of the blacklip site and in the same manner. The *H. laevigata* larvae were released on 7 December 1994, five days after spawning, and the inoculated sites were sampled twice, 6 days and 49 days after seeding. The two control sites were sampled 5 days after the day of seeding.

Later surveys

The greenlip site was later surveyed twice to estimate the density of survivors in the seeded area. At 153 days after seeding, we searched 16m^2 with a 1 m^2 quadrat by using an underwater magnifier *in situ*, and taking care to replace overturned rocks with minimal disturbance. At 335 days we searched an area of 10 m^2 which was estimated from the number of boulders searched; in this survey we selected boulders haphazardly throughout the entire seeded area.

Statistical analysis

To estimate the surface area of sampled boulders we weighed a subsample of 25 boulders and measured their individual surface areas (SA) by carefully covering each with a single layer of paper. After logarithmic transformation of the data on weight (W) and surface area of boulders, a least squares regression of SA in cm^2 vs W in g gave the equation:

$$SA=4.106\ W^{0.643}\ (R^2=0.98)$$

We then standardised the sample data to numbers of post-larvae m^{-2} surface area by using the above equation.

The data were analysed as a three factor (for *H. rubra*) and four factor (for *H. laevigata*) analysis of variance (ANOVA) in which release density and tent/no tent (and time for *H. laevigata*) treatments were fixed factors and micro-site was a random factor. Cochran's test showed that the untransformed data were significantly heteroscedastic ($p<0.05$) so we applied a $\sqrt{(x+1)}$ transformation which satisfactorily corrected the variance structure. We applied Dunnett's test (see Zar 1974) for the *H. rubra* seeding experiment to compare the treatment means with control means, and Tukey's test for comparison of treatment means.

Survival

The 6- and 49-day density estimates for *H. laevigata* were converted to numbers m^{-2} planar surface of the seabed for comparison with the later surveys expressed in the same units. The mean density of boulders at the greenlip site was 21.9 (s.e.1.4) m^{-2} (N=66), and the mean total surface area of boulders was 7028 (s.e. 576) $cm^2\,m^{-2}$. Assuming the underlying substratum was flat its area is 10,000 cm^2, giving a total mean surface area of about 1.7 m^2m^{-2}. So we multiplied the 6- and 49-day densites by 1.7 and then calculated survival. Estimates of larval survival in both experiments were obtained in the same way.

Results

Growth rates

The abalone larvae were about 250 µm long on release. After 19 days the modal mean for post larvae of *H. rubra* was 593 µm (range 400–950 µm) giving a mean growth rate of 18.1 µm day^{-1},

after pooling of the three density treatments which showed no significant differences in growth rate (t_{178}=0.6; ns). For *H. laevigata* the modal mean at 6 days was 433 µm (range 350–500 µm) and at 49 days was 1.06 mm (range 0.6–1.5 mm) giving a mean growth rate of 30.5 µm day^{-1} for the first 6 days and 14.7 µm day^{-1} for the next 43 days. The growth rates of the high and low density treatments did not differ significantly (t_{32}=0.02; ns). These mean growth rates are similar to those recorded by Rodda *et al.*(1997) for *H. laevigata* i.e. 19 µm day^{-1}, and only slightly less than those recorded by Tong *et al.* (1987) for *H. iris* i.e. 22 µm day^{-1} during the first 96 days after settlement.

H. rubra seeding experiment

Enhancement of densities of post-larvae was highest at intermediate (16,000 m^{-2}) release densities, compared with low and high release densities (Fig. 1), and significantly higher than controls (× 4.5) for both tent and no-tent treatments (Dunnett's test: tents q'=2.23, p<0.05; no tents q'=2.31, p<0.05). At low and high release densities only the tent treatments enhanced (x2.5) post-larval densities significantly above the controls (Dunnett's test: low density, tents q'=1.97 p<0.05; high density, tents q'=2.13 p<0.05). The ANOVA (Table 1) for *H. rubra* shows that both fixed factor treatments, density and tents, were significant, as well as the interaction between them. The interaction term was significant because the effect of tents which increased settlement densities at low and high larval release densities was reversed at intermediate release densities.

Pairwise comparisons between all treatments showed that only three treatments differed significantly (Tukey's test, p<0.05). With the treatments numbered 1–6 from the left in Figure 1, Nos 3 and 4 (intermediate density) were different from No.6, and Nos 2 and 4 differed.

A comparison of the mean proportion of surviving larvae in the three density treatments, after combining tent and no-tent treatments, shows that after 19 days the survival of released larvae was 1.9%, 0.5% and 0.03% for the low, intermediate and high density treatments respectively.

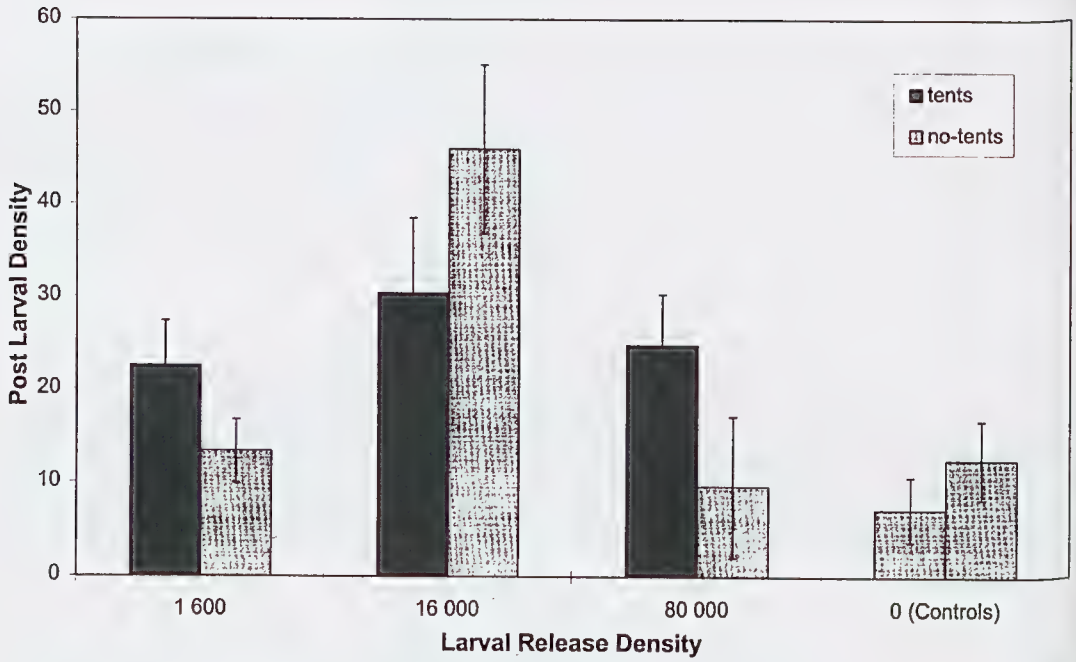

Figure 1. Mean densities of *H. rubra* 19 days after release at three release densities for tent and no-tent treatments. Vertical bars are standard errors. Post-larval densities are in numbers m^{-2} of surface area of substratum. Larval release densities are in numbers m^{-2} of the planar area of the substratum. The two control sites. Cathedral Rock and Shag Cove, were to the south and north respectively of the experimental site.

Table 1. ANOVA table for larval seeding experiments
** : p<0.01. * : p<0.05

Source of variation	*H. rubra* SS	df	MS	F-ratio	*H. laevigata* SS	df	MS	F-ratio
tents	9.49	1	9.49	20.8**	3.16	1	3.16	2.10
density	62.86	2	31.43	68.9**	16.49	1	16.49	10.99**
site	2.73	6	0.46	0.09	15.65	3	5.22	3.48*
time					197.29	1	197.29	131.49**
tents x density	21.61	2	10.81	23.7**	3.83	1	3.83	2.55
tents x time					1.38	1	1.38	0.92
density x time					7.35	1	7.35	4.90*
tents x density x time					0.39	1	0.39	0.26
tents x density x time x site					31.51	21	1.50	
residual	183.31	36			33.52	32	1.05	
total	280.02	47			310.57	63		

H. laevigata seeding experiment

No post-larvae < 500 µm were found at the two control sites at the 6 day sampling, so we attributed all the post-larvae found at the 6 and 49 days samplings to the seeding experiment. The ANOVA (Table 1) shows that density, sites and time were significant as well as the interaction term, time x density. The tent treatments resulted in higher average densities both at 6 and 49 days (Fig.2) but the differences were not significant due to the large within-site variability.

The mean proportion of larvae that settled and survived 6 days, after combining tent and no-tent treatments, was 6.5% in the low density treatment and 0.4% in the high density treatment.

Mean densities of settlers declined from 272 m^{-2} at 6 days (after averaging all treatments) to 6.5 m^{-2} at 49 days (Table 2), giving a mean survival of about 2.4% over 43 days. However, if the high and low density treatments are partitioned and survival estimated for each, survival in the low density treatment was 3.0 (s.e. 1.9)% and in the high density treatment was 2.1 (s.e. 1.1)%; these do not differ significantly. At 335 days overall survival of settlers was about 0.2% (Table 2).

Discussion

The *H. rubra* experiment took place against a background of natural abalone settlement. At a size of 400–1,000 µm we cannot distinguish newly settled post-larvae of the four species of abalone *H. laevigata*, *H. roei*, *H. rubra* and *H. scalaris* whose adults were present at the blacklip site and control sites. Our data could therefore have included post-larvae of any of these species. However, *H.*

Table 2. Mean density of post-larval/juvenile seed of *H. laevigata* at the greenlip site for 4 sampling periods. Densities are standardised to numbers m^{-2} of the planar area of the substratum. Survival values and instantaneous mortality rates (M) of settlers are given after 49 and 335 days. Standard errors in brackets.

	6 days	49 days	153 days	335 days
Mean density	272 (56)	6.5 (1.2)	0.61 (0.18)	0.63 (0.13)
Survival (%)		2.4		1.3
M mth^{-1}		2.6		0.4

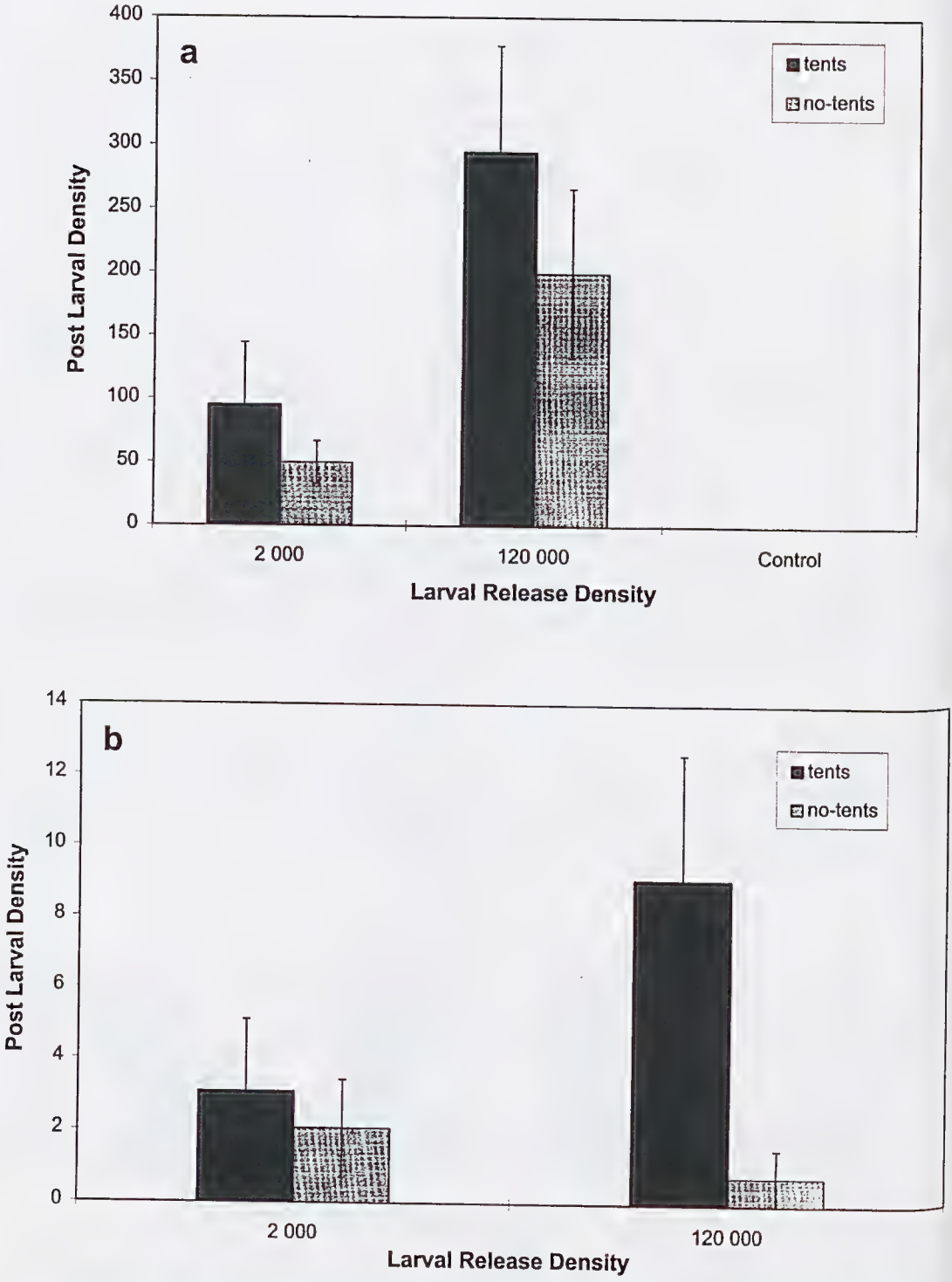

Figure 2. Mean densities of *H. laevigata* (a) 6 days, and (b) 49 days, after release at two release densities for tent and no-tent treatments. See Fig. 1 for meaning of density scales.

roei larvae settle only at 1–2 m depth (see Shepherd 1973, Shepherd unpublished data). *H. laevigata* and *H. scalaris* both spawn mainly from October–May (Shepherd and Laws 1974, Shepherd *et al.* 1986) so their post-larvae are unlikely to have been present during our seeding experiment in August. *H. rubra* spawns from July–September (Shepherd and Laws 1974, Rodda *et al.* 1997), and no spawning occurred at the date of our seeding experiment although minor spawning occurred at nearby sites in July 1994 (Rodda *et al.* 1997). We surmise that the background post-larval densities probably resulted from these minor spawning episodes of *H. rubra*.

Explanation of the results of both experiments must take into account the dispersal of larvae before settlement and post-settlement mortality. The fact that the tent treatments generally elevated post-larval densities above those of no-tent treatments indicates that larval advection from the sites, especially in no-tent treatments, must be substantial. The non-significance of the tent treatments in the *H. laevigata* experiments appears to be due to the great variability in the number of post-larvae found at 6 days. Besides the variability in larval loss, a variable micro-scale post-settlement mortality (Keesing and Halford 1992) seems very likely. The latter explanation would also account for the significant interaction between density and tent treatments in the *H. rubra* experiment.

Our survival estimates over 335 days are almost certainly low for the following reasons. First, diver efficiency must have been <100% at the 153- and 335-day samplings. Shepherd (1997) recorded only about 40% efficiency of divers at finding juveniles <20 mm long but that was in habitat where boulders were two layers deep whereas they were only one layer deep at this site. We expect our efficiency at this site to have been 60-70%. Second, some juvenile seeded abalone would have dispersed from the seeded island plots into the surrounding unseeded areas (c. 200 m^2) when the 153- and 335-day samplings were done. This dilution effect would cause an under-estimate of survival. Shepherd and Godoy (1989) recorded random movement of about 0.5 m mth^{-1} for 1-yr old *H. laevigata*, but we would expect less movement than this for younger ones. Thirdly, seeded larvae could have settled outside the plots in the surrounding areas so enhancing densities. Taking these considerations into account our survival estimate at 335 days (0.4%) is a minimal value and the true value could be as high as 1% of those alive at 6 days.

This paper is the first that considers post-larval survival of abalone at different initial densities, and so provides further evidence of density-dependent mortality (M) from settlement as previously shown by McShane (1991). Shepherd and Daume (1996) reported density-dependent M from two months after settlement, but their technique could not estimate M from the time of settlement. There are four other published studies of natural mortality of post-larval abalone in the first few months from settlement. McShane (1991) recorded M rates of 0.6–0.8 mth^{-1} for *H. rubra* in studies of natural settlement. Schiel (1992) seeded larvae of *H. iris*, and also used tents to retain them. About 10% of the larvae settled and subsequent M was 1.0–1.3 mth^{-1}. Sasaki and Shepherd (1995) found M of 1.4–2.7 mth^{-1} for *H. discus hannai* in a study similar to McShane's, and McShane and Naylor (1995) recorded M = 0.6 mth^{-1} in a seeding experiment also for *H. iris*. Our estimate of M=2.6 mth^{-1} for the same initial period of 2 months is higher than in Schiel's (1992) experiments and in the upper part of the range of the above published values, but is similar to that of many other gastropods (reviewed by Gosselin and Qian 1997).

Was mortality in the first 50 days from settlement due to intra-specific competition for bacterial and diatom food on CCA (Kawamura 1996) or was it due to predation or other factors (see Gosselin and Qian 1997)? Post-larval growth rates did not differ significantly between any of the density treatments for *H. rubra* or *H. laevigata,* implying that competition may be less important than predation during this early phase of the life history, at least over the range of densities tested.

Six months after settlement M declines sharply, although it still seems to be very variable. For this period Shepherd (1997) has recorded M in the range 0.1–3.2 yr^{-1}. So our maximum estimate of M 2.9 yr^{-1} for the period 49–335 days for *H. laevigata* is plausible. Our data for *H. laevigata* suggest that, if larvae are seeded at 2000 m^{-2}, about 0.03% of them will survive to one year. This assumes, conservatively, that larvae which did not settle in our experimental plots died. Thereafter, if we assume that M is 0.4 yr^{-1} from age 1 to 3, and 0.2 yr^{-1} from age 3 to 6 (Shepherd and Breen 1992), about 74 individuals per million seed would survive to 6 years, when harvested.

Both seeding experiments showed that the lowest density treatments produced the highest proportion of survivors when first sampled after settlement. Thus they imply density-dependent M. However, the evidence is inconclusive because the extent of larval loss from the plots before settlement is not known. If M proves to be strongly density-dependent at settlement then the best strategy for future seeding would be to seed at lower densities than those we have so far tested. The overall practical and economic feasibility of seeding in the light of our experience in these and related experiments will be considered in a later paper.

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Size-structure and growth of individuals suggest high exploitation rates in the fishery for blacklip abalone, *Haliotis rubra*, in New South Wales, Australia

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Abstract

The proportion of recent recruits in the catch of the NSW abalone fishery was estimated by combining information on growth rate and the size-structure of the commercial catch. Growth rates of individuals around the minimum length limit of 115 mm suggest most individuals less than 125 mm recruited to the fishery in the last year. These individuals make up between 50% and 95% of the commercial catch in all zones of the fishery. This dominance of recent recruits in the catch of the fishery makes monitoring of the abundance of pre-recruits, to provide information on likely fluctuations in recruitment, particularly important to management. Growth rates of individuals below the minimum length limit of 115 mm suggest most individuals above 100 mm will recruit to the fishery in the next year. Monitoring over the last four years has found no significant decline in the relative abundance of these pre-recruits in six major regions of the fishery. The high exploitation rate, suggested by the combination of patterns in growth and the proportion of recruits in the catch, combines with several other factors to make the NSW abalone fishery very different from those for the same species in other states in Australia.

Introduction

The recruitment of new individuals is one of the most important processes determining the dynamics of many marine populations (Underwood and Fairweather 1989). In populations exposed to fishing, two types of recruitment are often defined. Individuals can recruit to the population following settlement and metamorphosis (here referred to as settlement), and subsequently, as they grow, individuals can recruit to a fishery (here referred to as recruitment). Settlement and recruitment of many species varies greatly in time and space, and as this variation can have important consequences for the dynamics of the population, it can also have important consequences for management of any associated fishery (e.g. Shepherd and Partington 1995). Because a range of processes can operate to alter the patterns apparent at settlement (e.g. McShane 1991), the abundance of individuals immediately prior to recruitment is potentially much more important to management of a fishery.

The importance of variation in recruitment to a fishery is influenced by the size of the stock already recruited. For example, variation in recruitment becomes crucial when the fishery is dominated by new recruits (e.g. some species of prawn, Garcia and Le Reste 1981). Variation in recruitment becomes less important when the population available to the fishery is made up of a large number of accumulated age-classes that dominate the new recruitment (e.g. orange roughy, Mace *et al.* 1990). Populations of abalone are often portrayed as being dominated by an accumulation of large, old individuals (e.g. Sainsbury 1982, Breen 1986, Tegner *et al.* 1989, Nash 1992). These accumulations can be particularly vulnerable to fishing (Davis *et al.* 1996), and their depletion can lead to local settlement and recruitment failures and further decline in the population (Shepherd and Partington 1995).

In this paper we describe the apparent importance of recruitment to the New South Wales (NSW)

abalone fishery. We do this by combining information on the growth rates of pre-recruits (i.e. defined as those individuals that will recruit to the fishery within the next year) and recruits (i.e. defined as those individuals that have recruited to the fishery in the last year), with information on the length-structure of the commercial catch. These data indicate that the NSW abalone fishery is dominated by individuals that have recently recruited to the fishery. As a consequence, monitoring of the relative abundance of pre-recruits is likely to provide information on future recruitment, and hence the abundance of abalone available to the fishery. Finally, we compare the importance of recruitment to the fishery in NSW with its importance in fisheries for the same species in the more southern states of Victoria and Tasmania (Figure 1). It appears that differences among the states in the biology and ecology of abalone, the behaviour of commercial divers, and patterns of management have led to very different fisheries.

The commercial fishery for abalone (*Haliotis rubra*) in NSW is currently limited to a Total Allowable Commercial Catch (TACC) of 333 t. This is substantially lower than fisheries for the same species in Victoria (over 1500 t) and Tasmania (over 3000 t). In addition, there are also differences in the minimum length limit among the states. In NSW, there is a single length limit of 115 mm, whilst in the other states several different length limits are enforced. In Victoria most abalone are caught using a 120 mm length limit, whilst in Tasmania most are caught using a 132 mm length limit (see Prince and Shepherd 1992 for other differences in management among the states). In general, in each state commercial divers use trailable boats to gain access to sites. Fishing effort at a site is determined by a range of factors including its distance from a boat ramp, weather conditions and expected catch rates. Once at a site, divers will search an area of reef and collect most of the abalone they encounter over the length limit. Catch rates in NSW (about 20 $kg.hr^{-1}$) are much lower than in the southern states (above 50 $kg.hr^{-1}$), due mainly to the additional time required to find abalone above the length limit.

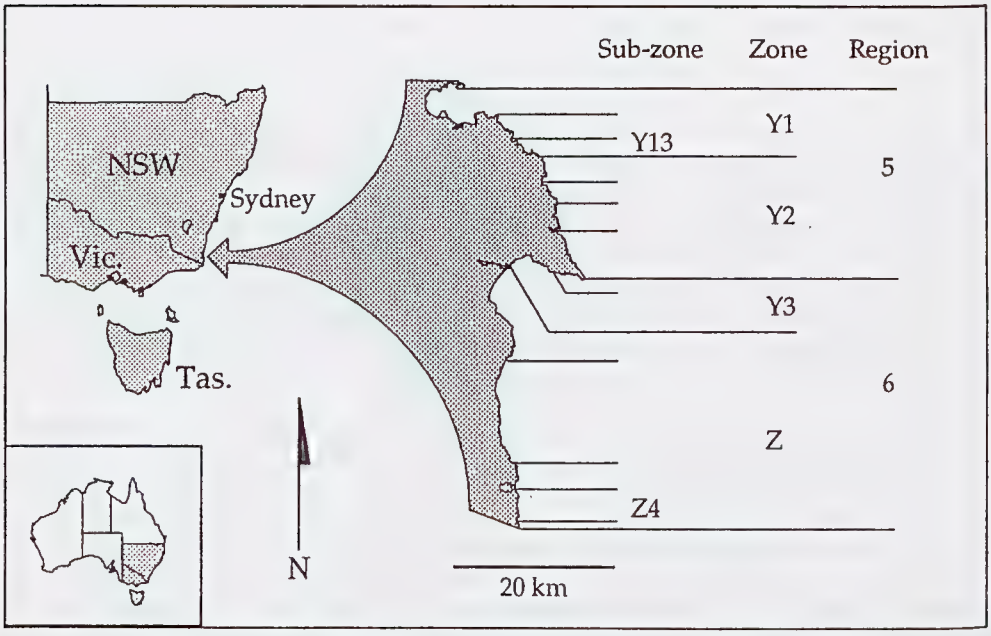

Figure 1. Map of Australia (inset) showing states of New South Wales (NSW), Victoria (Vic.) and Tasmania (Tas.), with section of NSW coast showing sub-zones, zones and regions described in text.

Materials and methods

Growth of pre-recruits and recruits

Estimates of growth were derived from the tagging data set described in Worthington *et al.* (1995). Briefly, maximum likelihood was used to fit a model containing up to six parameters to the length-increment data. One of the parameters described the coefficient of variation in growth among individuals (see Francis 1995). This allowed estimates of the expected growth, and its confidence intervals, for an individual of a given length. For example, at one site (Eden II) the expected annual growth of a 90 mm abalone was 19.9 mm and the 95% confidence interval was between 4 mm and 35 mm. In other words, 95% of 90 mm abalone would be expected to be between 94 mm and 125 mm one year later.

Estimates of growth and its variation can also be expressed in terms of the proportion of individuals expected to reach a given length. In the example above, about 25% of 90 mm abalone will grow more than 25 mm in a year and enter the fishery. This allows the calculation of the proportion of abalone of a given length that will enter the fishery within one year. As estimates of growth and variation in growth among individuals will vary among sites (Worthington *et al.* 1995), we have calculated the percentage of individuals of a given length that will grow to ≥115 mm and enter the fishery within the next year for both a fast and a slow growth site (Eden I and Eden II in Worthington *et al.* 1995). In addition, we have calculated the proportion of individuals of a given length that will grow to ≥125 mm within the next year. Rates of growth suggest that most individuals ≥125 mm would have been recruited to the fishery for more than one year (see later).

Size-structure of the commercial catch

Estimates of the size-structure of the commercial catch were gained by measuring a sample of abalone from a subset of the daily catches collected by commercial divers. When logistically possible an abalone processor is visited, and for all divers and days available, the shell length, width and total weight of a sample of abalone were measured to the nearest mm or 5 g. Only one bin (about 50–100 individuals) per diver per day is sampled. This sampling design was chosen because the size-structure of abalone in different bins from the same diver on the same day is more similar than between bins from the same diver on a different day or among different divers (Andrew and Chen 1997). The sampling of only 50–100 abalone per diver per day allowed us to sample approximately 100 diver-days out of a total of about 3000. This sampling design should provide a precise estimate of the size-structure of the commercial catch (Andrew and Chen 1997).

The size-structure of the commercial catch has been estimated in this way for each year since 1993, and some comparable estimates are available from earlier years. The comparison among years is complicated by differences in the minimum length limit. A minimum length limit was first introduced in 1973 (100 mm) and was increased in 1980 (108 mm), 1986 (111 mm) and 1987 (115 mm). Since 1993, estimates of size-structure are available for six regions in the fishery which are used for stock assessment purposes (Figure 1). Estimates are also available for a selection of zones and sub-zones in the fishery (Figure 1). These smaller spatial divisions are used for the reporting of daily catch and effort statistics by the commercial divers, and represent the smallest possible scale for management that is currently available.

Relative abundance of pre-recruits

During surveys of the relative abundance of abalone at fixed sites spread along the NSW coast, estimates of the size-structure of the populations were also collected. These surveys were started in 1994 and have been repeated in 1995 and 1996 between June and December each year. At more than 40 sites, all abalone encountered were gathered by carefully searching all caves, crevices and boulders within an area of about 20 x 20 m until a sample of approximately 100 was collected. No boulders were over-turned during this process. The abalone were brought to the surface where the shell length and width of each were measured to the nearest mm. Abalone were then returned to the water, and placed by hand in the area from which they were collected. Only the relative frequency of individuals >90 mm in length is shown here, as few individuals below this size can be considered pre-recruits.

Results

Growth of pre-recruits and recruits

Rates of growth differ considerably among sites within NSW (Worthington *et al.* 1995). This is reflected in the proportion of abalone of a given length entering the fishery within one year (Figure 2). For example, at sites where individuals grow quickly, more than 50% of 100 mm abalone are pre-recruits, and more than 25% of 90 mm abalone are pre-recruits. In contrast, less than 15% of 100 mm abalone are pre-recruits at sites where they grow slowly, and it is not until almost 110 mm that more than 50% of individuals are pre-recruits. Because more individuals are collected from sites where the abalone grow quickly (see Worthington and Andrew 1997), it is likely that the average proportion of pre-recruits at a length is more closely related to that at sites where individuals grow quickly rather than sites where they grow more slowly.

Variation in growth among sites is also reflected in the proportion of individuals growing to ≥125 mm within one year (Figure 2). Less than 10% of 90 mm individuals will grow to ≥125 mm at both fast and

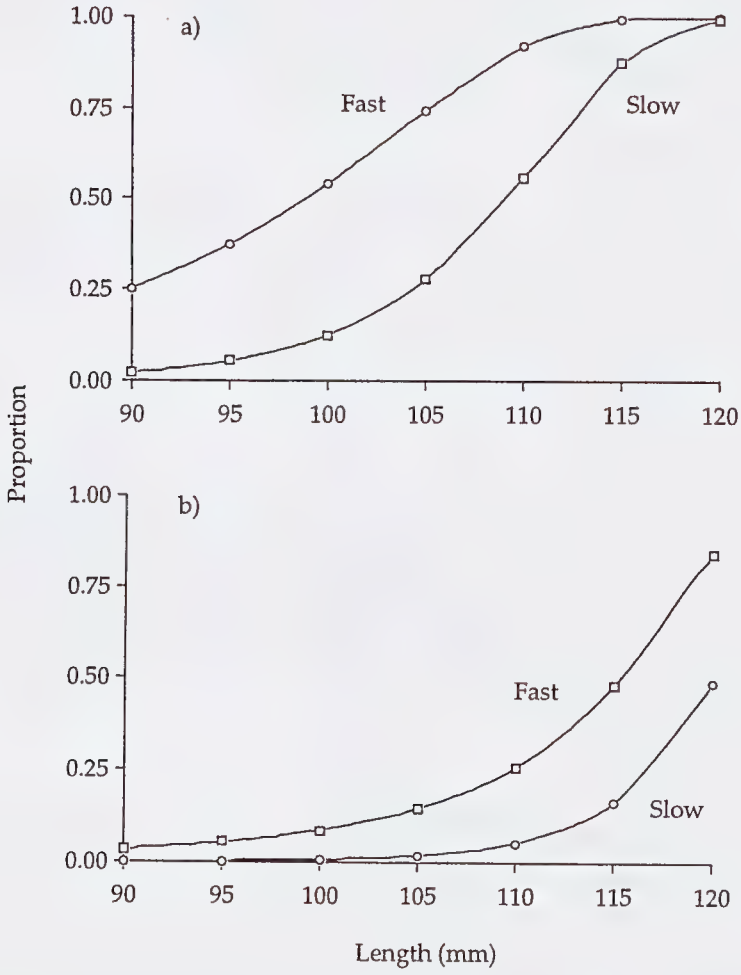

Figure 2. Proportion of abalone reaching a) 115 mm in length and b) the length-classes in the fishery >125 mm, after one year plotted against their starting length for a fast and slow growth site.

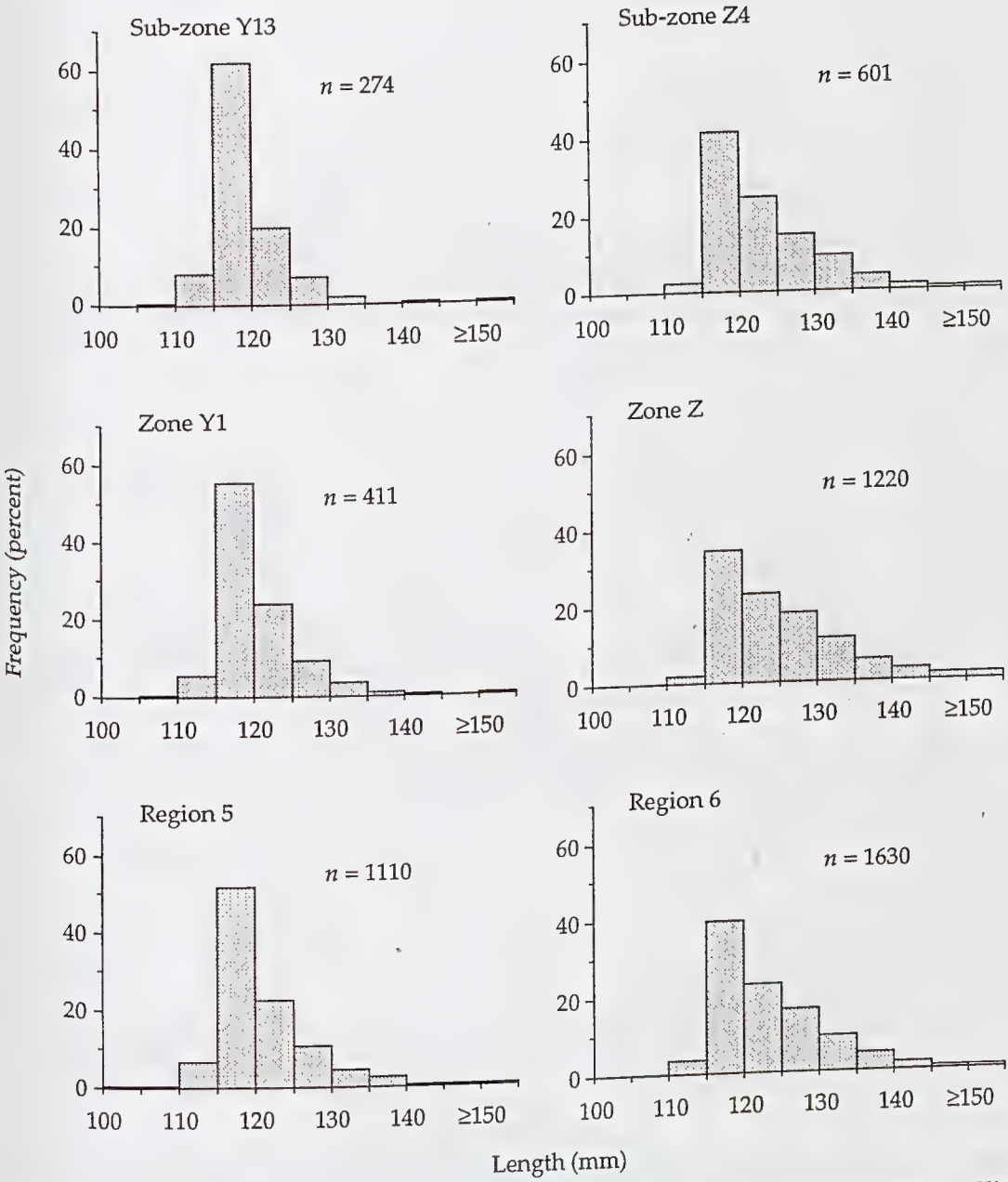

Figure 3. Length distribution of the commercial catch of abalone during 1996 from sub-zones Y13 and Z4, zones Y1 and Z, and regions 5 and 6. Sample sizes are also shwon.

slow growth sites. Even by 120 mm, only 50% of individuals will reach 125 mm within one year at sites where they grow slowly. For 115 mm abalone, about 50% of individuals at fast growth sites, and 20% of individuals at slow growth sites, will reach 125 mm within one year.

Size-structure of the commercial catch

During 1996, there was significant spatial variation in the size-structure of the commercial catch at

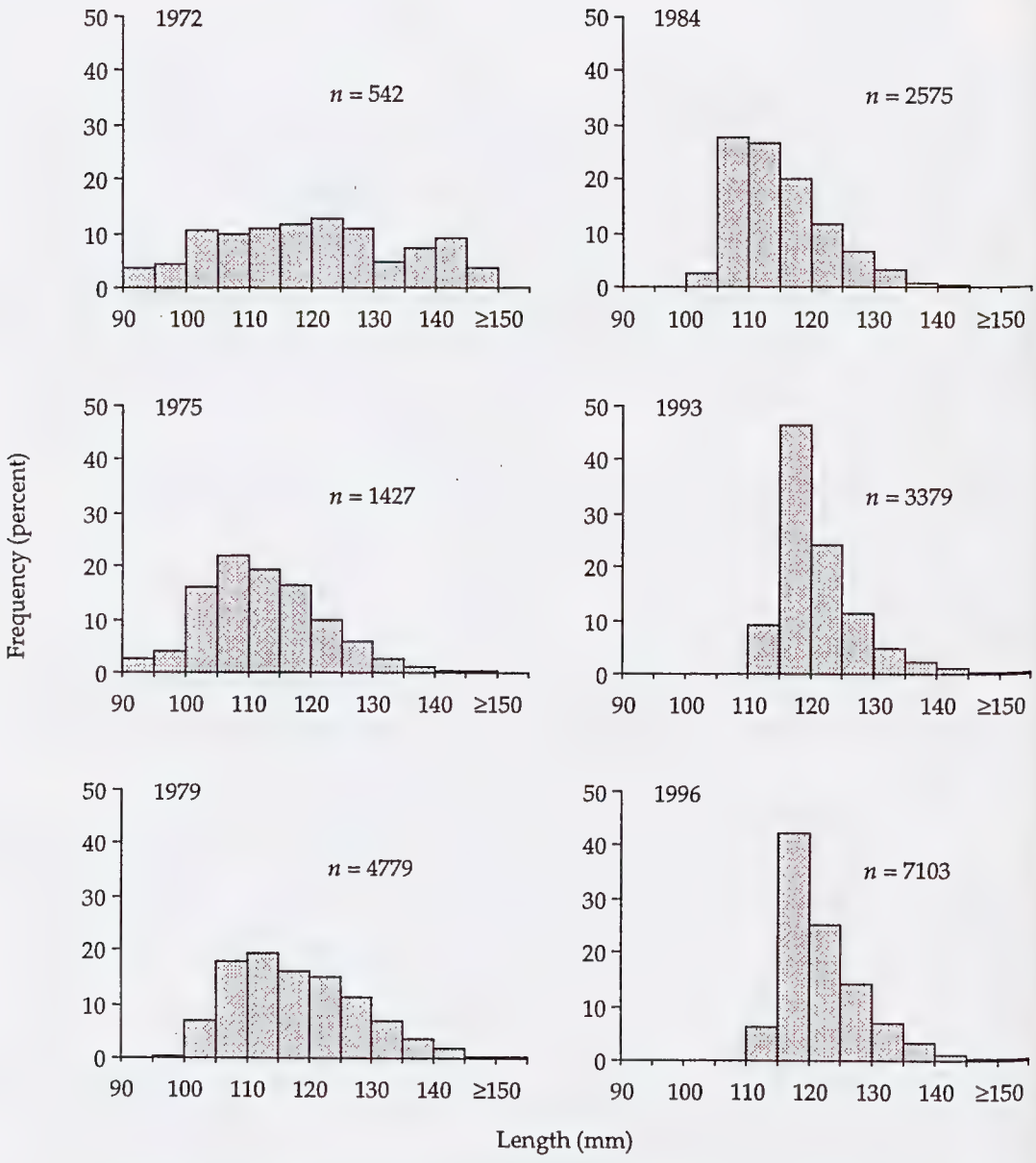

Figure 4. Length distribution of the commercial catch of abalone in NSW during several years. Note, there was no restriction on the minimum length of abalone during 1972, but a 100 mm length limit was enforced in 1975 and 1979, 108 mm in 1984, and 115 mm in 1993 and 1996. Sample sizes are also shown.

a range of spatial scales. While the distributions declined relatively smoothly away from the minimum length limit in all samples, there were differences in the rate of this decline. For example, there was a steep decline (slope of regression (± s.e.) = 0.18 ± 0.04) in the length-distribution from sub-zone Y13 (Figure 3), and most individuals caught were relatively close to the length limit (Figure 3). Over 90% of individuals caught in sub-zone Y13 were <125 mm in length. In contrast, the distribution from sub-zone Z4 (Figure 1) was comparatively flat (slope of exponential decline = 0.12 ± 0.01), and only 68%

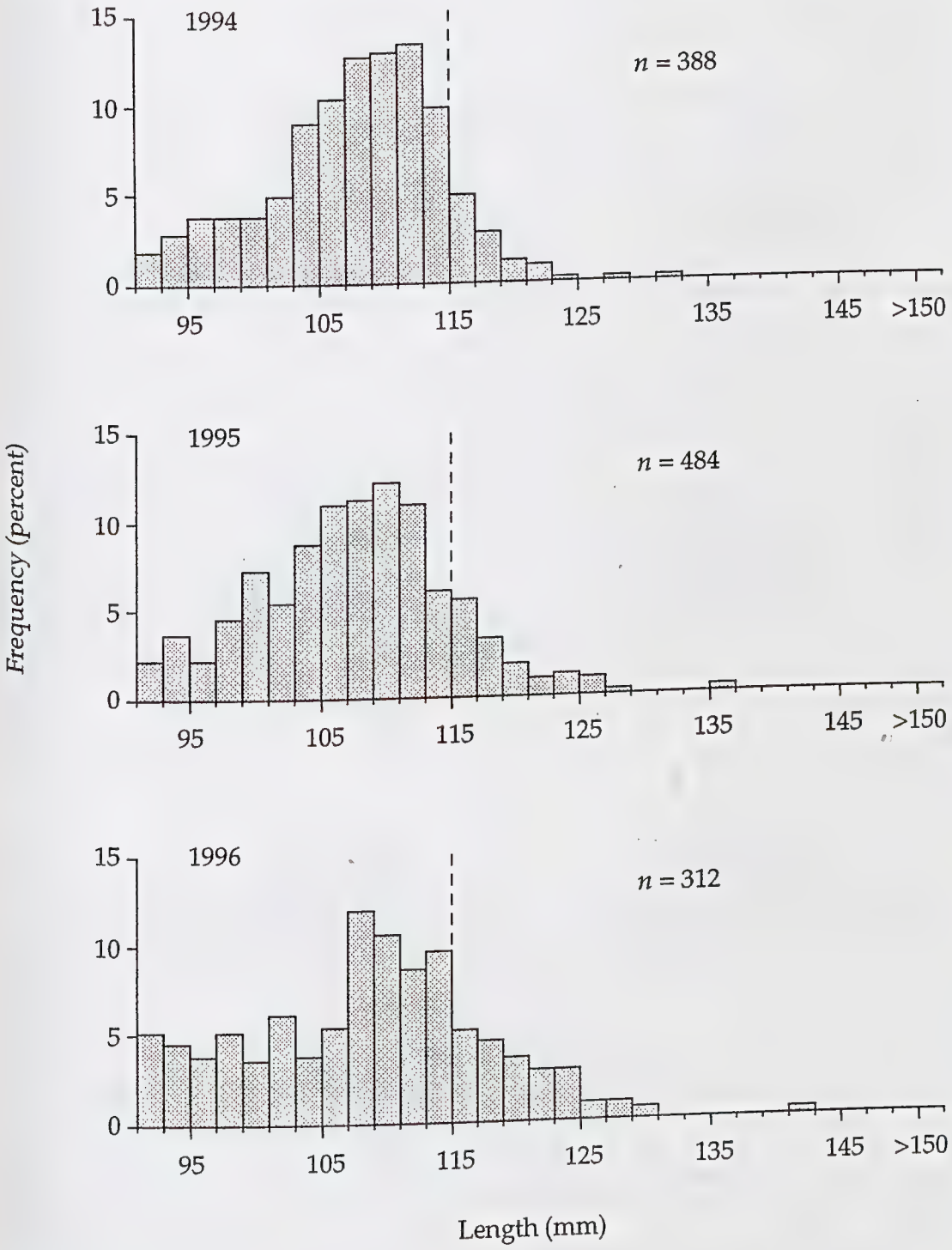

Figure 5. Length distribution of abalone populations furing 1994, 1995 and 1996 in region 4. Sample sizes are also shown.

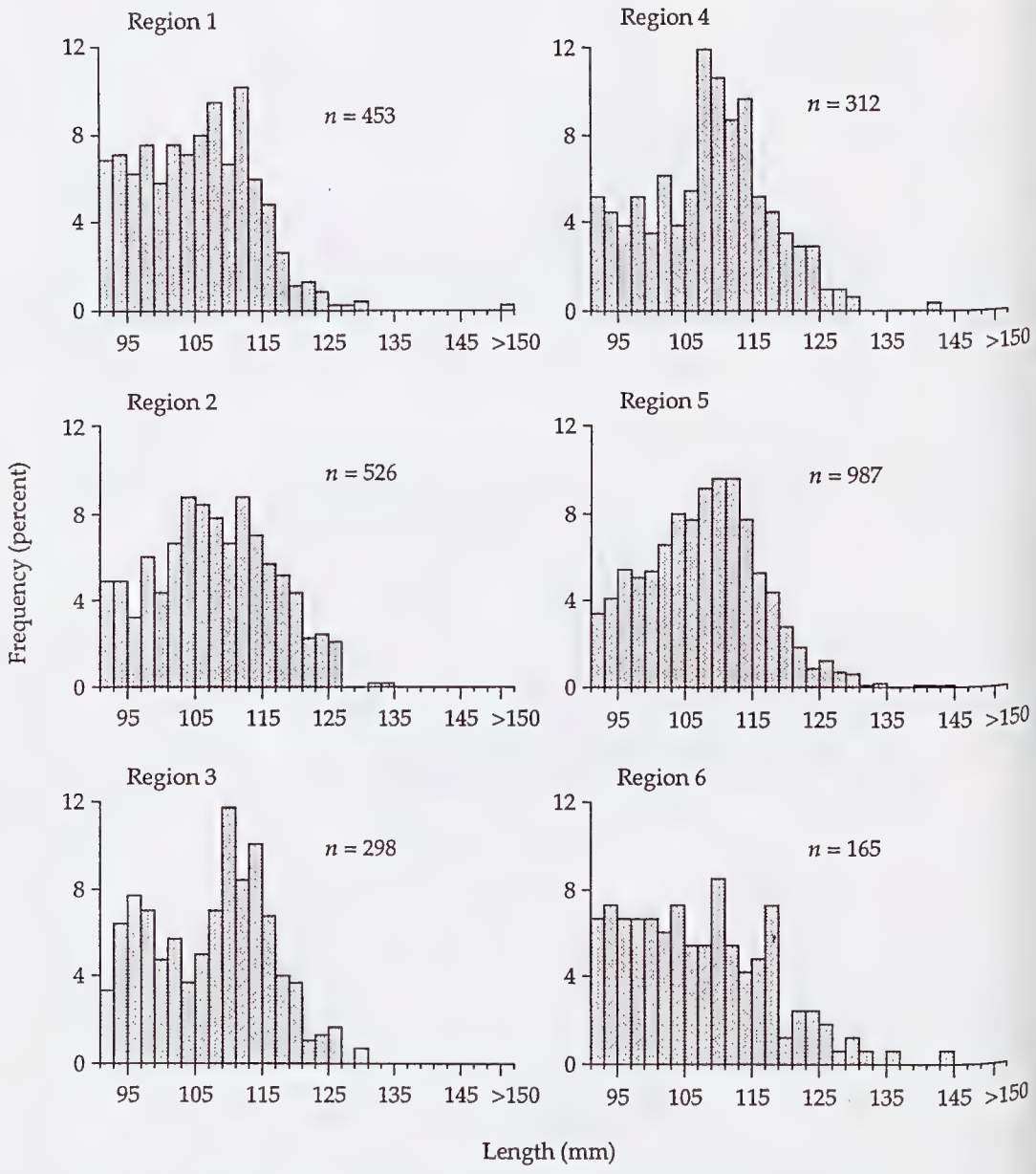

Figure 6. Length distribution of abalone populations during 1996 in each of six regions. Sample sizes are also shown.

of individuals were below 125 mm in length (Figure 3). At the larger spatial scales of zone and region, similar patterns were also evident. More larger individuals were caught in zone Z and region 6 than in zone Y1 and region 5 (Figure 3). In region 5, over 80% of individuals caught were <125 mm, whilst in region 6 this dropped to 65%.

During the last four years, there has been a small decline in the proportion of individuals <125 mm in the catch of the whole fishery. For example, during 1993 80% of individuals were <125 mm in length, whilst in 1996 this dropped to 74% (Figure 4). This reduction is likely to represent a significant change

in the length structure of the population (Andrew and Chen 1997). The proportion of individuals in these length-classes during the last four years is much larger than in previous years, although this comparison is complicated by differences in the minimum length limit. For example, during 1972 there was no minimum length, and individuals were much more evenly spread among the length-classes above 90 mm (Figure 4). During 1975 and 1979, a 100 mm minimum length limit was enforced and individuals were less evenly distributed among the length-classes, but the length-class immediately above the limit was not the most common in the catch (Figure 4). During 1984, when a 108 mm length limit was being enforced, individuals were still more evenly spread among the length-classes than in the last four years (Figure 4).

Relative abundance of pre-recruits

Patterns in the size-structure of abalone in the population were consistent with relatively fast rates of growth. For example, in region 4 during 1994 only 11% of individuals >90 mm in length were also =115 mm, and hence already recruited to the fishery (Figure 5). Individuals between 105 mm and 114 mm were relatively abundant. By 1996, the proportion of individuals immediately below the length limit had declined, and over 22% of individuals >90 mm in length had recruited to the fishery at that time. That is, the strong cohorts of pre-recruits seen in 1994 and 1995 had recruited to the fishery in 1996, and led to a large increase in the proportion of individuals available to the fishery. This is supported by both observed increases in the catch rate of commercial divers, and in the abundance of individuals =115 mm in the independent surveys (Andrew *et al.* 1996).

Spatial variation in the relative abundance of pre-recruits is likely to be reflected in future patterns in catches. For example, in region 6 during 1996, 24% of individuals >90 mm were already recruited to the fishery, and relatively few individuals were immediately below the length limit (Figure 6). In contrast, in region 3 during 1996, there were comparatively few individuals already recruited to the fishery (19%), but two strong cohorts were present immediately below the length limit (Figure 6). Abundances of individuals <115 mm have also increased over the last two years of the independent survey in this region. Many of these individuals are likely to recruit to the fishery in the next year. In contrast, in region 1 during 1996 only 12% of individuals >90 mm were available to the fishery (Figure 6), and abundances of individuals <115 mm had declined in the independent surveys. Recruitment to this region over the next year is likely to be lower than all other regions.

Discussion

Recruitment to the NSW abalone fishery

A large proportion of individuals caught in the NSW abalone fishery have recruited within the last year. This proportion appears, however, to vary in space within the fishery, and is related to variation in demographic factors such as patterns of settlement, growth and mortality. When strong year-classes grow quickly and become available to the fishery in zones where few individuals are already above the length limit, up to 95% of the commercial catch can be <125 mm in length. In other zones with lower exploitation rates and more larger individuals, individuals in these length-classes can still often represent over 40% of the commercial catch (Andrew and Chen 1997). In much smaller areas (i.e. <1 km) within some sub-zones, slow growing, stunted populations exist (Worthington *et al.* 1995) that are dominated by accumulations of old abalone. Recent recruits are likely to represent a small proportion of the catch from these populations. Total catches from such slow growing populations are small, and populations that grow faster dominate the catch (see Worthington and Andrew 1997 for further discussion of the relationship between growth rate, frequency of fishing and total catch). Across the whole fishery, more than 70% of the total catch has been <125 mm in length over the last four years. Estimates of growth suggest most of these individuals have recruited to the fishery within the last year.

The proportion of recruits in a fishery is not only determined by demographic factors controlling the number of recruits, but also by factors affecting the individuals already available to the fishery. Perhaps

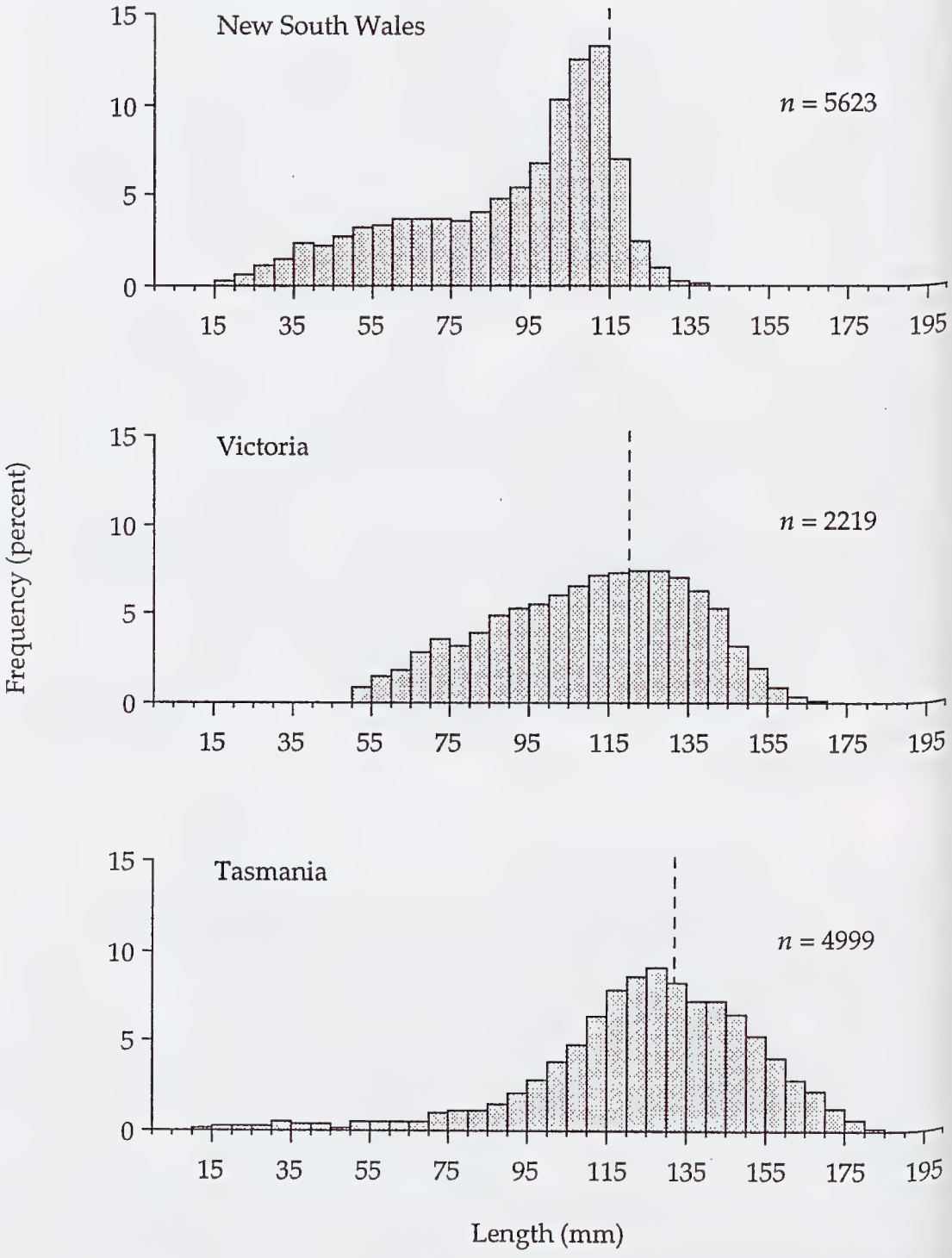

Figure 7. Length distribution of abalone populations during 1995 in New South Wales, Victoria and Tasmania. Sample sizes are also shown.

the most important of these include the effects of fishing and changes in the minimum length limit. The extent to which these factors are responsible for the large number of recruits in the NSW abalone fishery is unclear. Historic changes in the minimum length limit have certainly been responsible for changes in the number of individuals available to the fishery. This has been clearest following the large increases of the length limit in 1980 (100–108 mm) and 1986–1987 (108–111–115 mm). The effects of fishing on the number of recruited individuals, and hence the proportion of recruits in the fishery, is less clear. Possible trends are suggested by comparing the current size-structure with that from earlier in the fishery's development. Such comparisons suggest that there has been a significant change in the size-structure of the population. Perhaps most importantly, this suggests the accumulated stock of old, large abalone has been seriously depleted over the life of the fishery. It is important to note, however, that small increments in the minimum legal size limit over this period will complicate interpretation of these trends. Such a depletion is also supported by a comparison of the length-structure of abalone in NSW with those in Victoria and Tasmania where there are many more large (>125 mm) abalone (Figure 7). Consequently, it appears that the domination of recent recruits in the catch of the NSW abalone fishery is at least partly caused by a high exploitation rate.

The large proportion of recruits in the catch of the abalone fishery in NSW has several important consequences. Perhaps most importantly, it suggests that the fishery is no longer supported by an accumulation of large abalone. Rather, it is heavily reliant on the growth and recruitment of new individuals each year. This makes the fishery more vulnerable to failure in recruitment, as there is no opportunity for its effects to be dissipated by an accumulated biomass of larger individuals. Such a pattern has preceded further declines in several other abalone fisheries (e.g. Tegner *et al.* 1989). In contrast, in NSW indices of biomass, abundance and size-structure have been increasing over each of the last four years.

Potential for monitoring pre-recruits

The large proportion of recruits in the NSW abalone fishery also has a fortunate consequence for management. Knowledge of the abundance of pre-recruits can provide information on the likely future abundance of recruits and hence, of abalone available to the fishery. This enables more reliable forecasts of potential harvest when setting the TAC (see Walters 1989). Similar knowledge of patterns in the abundance of individuals at settlement (Nash *et al.* 1995), as pre-recruits (Farlinger and Campbell 1992) or at some intermediate age (Shepherd and Partington 1995), is used to aid management of other abalone fisheries. The ability to estimate patterns in abundance of individuals as soon as possible to when they recruit is clearly desirable, as the patterns are more likely to be modified as the intervening time increases. Current research in NSW is now concentrating on gaining a better understanding of these processes in the early life-history of abalone.

A variety of different methods could be used to estimate patterns in the relative abundance of pre-recruits. The size-structure of populations is sampled in areas adjacent to the independent surveys, where individuals are counted in length-classes but not disturbed, so the information from the two methods can be combined. By combining the independent information within small areas we hope to provide a more reliable estimate of patterns in the abundance of pre-recruits at this scale. Further, by combining patterns in relative abundance and size-structure from several small areas, estimates of the likely recruitment of individuals within a sub-zone, zone or region can be made. When localised recruitment failures occur, there is the opportunity to prevent further collection of individuals from certain sub-zones, as has been done in several sub-zones adjacent to Sydney (Figure 1). It remains to be seen whether recruitment returns to levels comparable to those before the closure.

A comparison with fisheries in other states

We have described above some of the similarities and differences between the fisheries for *H. rubra* in NSW, Victoria and Tasmania. Rates of growth, mortality and fecundity appear largely similar among the states (see Worthington and Andrew 1997). In contrast, perhaps the most conspicuous difference between the states is the apparent exploitation rate of the fisheries, as suggested by the length-structure

of abalone that remain (Figure 7). Other differences among the states also exist, including historical changes in management, patterns in the behaviour of divers, and the existence in NSW of the large, diadematid sea urchin *Centrostephanus rodgersii* which leads to fundamental differences in ecology of the associated reef (see Andrew and Underwood 1992).

The differences among the states described above have led us to question the appropriateness of some arguments derived from research on fisheries in the other states to the fishery in NSW. For example, in NSW divers mostly launch trailable boats to gain access to sites. Boat ramps are spread evenly along the coast, so that few sites can not be accessed by a short (<30 min.) boat trip. This combines with the high exploitation rate of the fishery to ensure that most sites where abalone exist are fished very frequently (i.e. on average, every sub-zone in the fishery is visited by a commercial diver at least once per week) and relatively few abalone are above the length limit at each site. This contrasts with areas in the other states, and with the inaccessible, west coast of Tasmania in particular, where stocks of large abalone exist. As a consequence, the arguments concerning serial depletion of stocks, that have evolved in Tasmania (e.g. Harrison 1983), have less appeal in NSW. This is not to say serial depletion is not occurring in NSW. Effort from the closed sub-zones adjacent to Sydney appears to have been shifted to the most southern sub-zones where some larger abalone exist (see Zone Z in Figure 3). Rather, serial depletion of stocks is mostly occurring on a much shorter time scale, equivalent to the high rates of visitation to most sites in the fishery. As there are no areas with stocks of large abalone remaining in NSW, the minimum length limit effectively prevents the further depletion of sites. Divers then choose to visit new sites before continuing to deplete the density of abalone in already heavily fished areas. Patterns in catch and effort still need to be carefully monitored to ensure this situation does not change.

Research in other states has also been used to suggest that the catch rate of commercial divers is a poor index of change in relative abundance of abalone (e.g. Prince and Shepherd 1992). Arguments supporting this are related to several factors. These factors include the potential of serial depletion to maintain divers' catch rates while stocks decline, the ability of abalone to actively aggregate as adults, and the reduction in catch rate at very high densities related to the handling time of individual abalone. As described above, in NSW the high visitation and exploitation rates suggest to us that at the moment serial depletion is not seriously biasing catch rate as an indicator of annual changes in relative abundance. In a similar way, the results of several tagging experiments in NSW provide no support for the argument that adult *H. rubra* actively move towards each other (G. Hamer, unpublished data). Finally, problems in analysing commercial catch data related to handling time simply do not occur at the low densities of abalone and subsequent catch rates that occur in NSW. As a consequence, we believe that standardised catch rates of divers in NSW currently provide a more reliable picture of annual change in the relative abundance of abalone than in the other states. Similar arguments were also used by Geaghan and Castilla (1986) to suggest catch rates of loco in Chilé are useful indicators of abundance.

We do not imply that catch rate will provide completely accurate and precise information on changes in the abundance of abalone in NSW. Rather, the NSW abalone fishery appears to present a situation where catch and effort statistics can provide useful information about the population, particularly when interpreted in conjunction with other measures. Indeed, the true worth of catch and effort can only be confirmed by comparison with an independent measure of abundance (e.g. abundances in the independent survey). These unique attributes of the NSW abalone fishery, which include the high exploitation suggested by the proportion of recruits in the catch, emphasise how different it is from fisheries for the same species in other states of Australia.

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Review of abalone culture and research in New Zealand

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Abstract

Despite interest and research in abalone aquaculture since the 1970s, the abalone farming industry is still in a nascent stage in New Zealand. The current aquaculture industry is based on mussels, salmon and rock oysters, and only trial marketing of cultured abalone has been done. The research on abalone in New Zealand reflects a young industry, with the largest category of published papers since 1990 on spawning and settlement. The status of the industry and research related to solving critical problems are reviewed. The major areas requiring more research are in overall system development and integration with food and stocking densities, food development both for recent settlers and later stages to achieve greater growth rates and lower variability among abalone, and algal harvesting effects and techniques to provide the information necessary for more harvesting permits to be granted. The industry will have to be increasingly focused on marketing and product development.

Key words: Abalone, *Haliotis*, aquaculture, New Zealand, Review

Introduction

As in most countries with a history of abalone fisheries there is considerable and increasing interest in abalone aquaculture in New Zealand. The reasons for this broadening of the production base from capture fisheries to farming have been discussed or mentioned in many publications and relate generally to market awareness and demand, coupled with occasional management problems and limits to the wild catch (Shepherd *et al.* 1992, Fleming and Hone 1996). In terms of research, there has been a change in emphasis during the past seven years. Fleming and Hone (1996) pointed out that 25% of papers presented at the First International Symposium on Abalone in 1989 related to abalone culture, whereas 50% of papers related to this topic at the second symposium in 1994. While this is not necessarily an accurate reflection of the overall research effort on abalone worldwide, there has clearly been more research published recently on abalone culture from a wide range of countries. The papers from the Second International Symposium on Abalone show there are common themes that encompass abalone research but also many factors unique to each country. This paper discusses some of these themes relating to abalone aquaculture and suggests how research in New Zealand can help the nascent industry and where it fails to solve industry problems.

The primary premise of this review is that the main purpose of aquaculture is to make money. The corollary of this is that all research should be directed primarily at solving specific problems that hinder the industry in development and production. This is especially important where research dollars are few and a form of funding triage occurs: the most needy problems should be identified and solved first. Four arguments are the focus of this review: 1) that research into abalone culture should have as a major component an assessment of the costs associated with developing techniques;

2) that research should be aimed at specific problems impeding progress in establishing viable aquaculture enterprises; 3) that the scale of experimental research and development should be appropriate to a commercial scale; and 4) that research should be appropriable by industry.

Background to abalone research in New Zealand

Three categories of background information are relevant to understanding the environment for abalone research in New Zealand. First, the abalone catch from the natural fishery has been relatively stable at around 1200t per year for the past decade (Schiel 1992a). There is considerable knowledge about the biology and ecology of the main fished species, *Haliotis iris* Martyn (1784), including feeding and movement (Poore 1972a, b), growth and mortality (Poore 1972c, Sainsbury 1982), morphological variation both locally and geographically (McShane *et al.* 1994a), reproduction (Poore 1973, Wilson and Schiel 1995, Hooker and Creese 1995), and fine-scale distribution (McShane *et al.* 1994b, McShane 1995). There has been considerably less research on the other two New Zealand haliotids, *H. australis* Gmelin (1791) and *H. virginea* Gmelin (1791).

Second, the aquaculture industry is not large in New Zealand and is based on only a few species (Table 1). There are 65,000t of the green-shell mussel *Perna canaliculus* produced annually on long-lines, of which 21,000t is exported with a value of NZ$87 million. Salmon, almost all of which is chinook (*Oncorhyncus tshwaytscha*) grown in sea-cages, has a total annual production of 6,500t, of which 5,000t is exported with a value of $34 million. Around 4,800t of the Pacific oyster *Crassostrea gigas* are grown annually, of which 1,436t is exported. Although there has been considerable discussion about aquaculture in New Zealand, and the pros and cons of indigenous species have been analyzed (Hayden 1988), little has translated into production. The aquaculture of paua has been discussed and researched for at least 18 years but, to my knowledge, only trial marketing of products and a small trade in production of larvae and juveniles have occurred.

Third, there are few sources of funds for aquacultural research in New Zealand. The Foundation for Research, Science and Technology (FoRST) provides most of the funding for all institutional research through its Public Good Science Fund. One output area is Fisheries and Aquaculture Industries, which has an allocation of $6.5 million (1996–97 figures), of which $1.5 million was specifically allocated for aquaculture production in 1996–97 (Anonymous 1996b). A few aquaculture research programmes are funded by industry, mostly with matching funds from a scheme administered by FoRST. Altogether, paua research has around 22% of the FoRST aquaculture funding, with projects on larval settlement and enhancement of natural populations, while industry contributes to others relating to food, algal harvesting, system development and pearl growth.

There has been a considerable amount of work on spawning, culture and rearing of paua since the late 1970s and there is continuing enthusiasm shown by prospective industry participants in attending hands-on workshops in paua culture. Given this work and interest over many years, it seems reasonable to ask what has resulted from this effort, interest and expenditure. Why is there not

Table 1 Aquaculture production in New Zealand for 1995, including export tonnage, value, and total production (domestic + export). Source of export figures: New Zealand Fishing Industry Board (Anonymous 1996a); salmon figures from N. Boustead (National Institute of Water and Atmospheric Research, pers. comm.). NZ$1 = US$0.70 (at November 1996).

Species	1995 export figures		Total
	t	$(million)	t
Oysters (*Crassostrea gigas)*	1,436	11	4,800
Salmon (mostly *Oncorhynchus tschawytscha)*[1]	5,061	34	6,500
Mussels (*Perna canaliculus*)	21,258	87	65,000

[1] Sockeye salmon (*O. nerka*) have a total aquaculture production of 10–20 t annually

more than an incipient industry in New Zealand? Has the research been appropriate for the production of paua and development and expansion of the industry, or are there other factors primarily responsible for what can be seen as slow progress?

Research into paua culture

As evidenced by the papers published from the Second International Symposium on Abalone, six major topics encompass most of the research effort in abalone culture internationally (Table 2). Artificial diets and nutrition have assumed considerable importance in the past few years, primarily because of the need for high quality foods that are predictably available. Various aspects of husbandry, including the use of growth hormones (Taylor *et al.* 1996) and anaesthetics (White *et al.* 1996) were presented, while there were two papers on system design. The problems and opportunities of marketing were discussed in one paper (Oakes and Ponte 1996). New Zealand research on paua produced 25 publications since 1990, 64% of which were related to fisheries and 36% to aquaculture (Table 2B). In comparing the subjects of the 11 aquaculture papers to the spread of topics internationally, only one was on diet and nutrition (Stuart and Brown 1994). The major categories for New Zealand research were spawning and settlement (36%; Moss and Tong 1992a, b, Moss *et al.* 1995, Moss In Press), enhancement of natural populations using hatchery-raised paua (18%; Schiel 1992b, 1993), and analysis of stress on paua due to handling and shipping (18%; Baldwin *et al.* 1992, Wells and Baldwin 1995). Other categories were system design and techniques (Tong and Moss 1992), and genetics relating to hatchery production (Smith and Conroy 1992). It therefore appears that the research effort in New Zealand has a different focus from research in other countries. Does this reflect different problems in New Zealand, a different stage of development of the industry, or something else? To answer these questions I categorise the problems in New Zealand paua farming and outline what is known. Almost all of this relates to *Haliotis iris*, although some interest in other species is developing (*e.g.*, Moss 1997).

Techniques for spawning and settling

The techniques for spawning paua are straightforward and routine, and similar to those used elsewhere (Hahn 1989, Tong and Moss 1992). In both *Haliotis iris* and *H. australis* gonads are at

Table 2 The number and percentage of papers published on selected aquaculture topics, A. from the Second International Symposium on Abalone 1994 (from Aquaculture 140, 1996), and B. since 1990 from abalone research in New Zealand.

A. From Second International Symposium on Abalone, 1994

Topic	Papers	%
Artificial diets and nutrition	8	50
Post larval culture	1	6
Husbandry	3	19
Tropical abalone	1	6
System design	2	12
Marketing	1	6

B. From New Zealand research since 1990

Topic	Papers	%
Diet and nutrition	1	9
Handling stress	2	18
Spawning/settlement	4	36
System design/techniques	1	9
Enhancement	2	18
Genetics	1	9

their smallest during late summer (Feb – Mar), although there is considerable local and regional variation (Poore 1973, Wilson and Schiel 1995, Hooker and Creese 1995). Paua can usually be spawned in all but the warmest summer months. Settlement is usually done on diatom-covered panels, without the use of GABA (c.f. Morse 1992). The techniques used throughout New Zealand are mostly those contained in a technical report (Tong *et al.* 1992), which is used in workshops for training potential farmers in paua culture.

Early settler survival and growth

The growth and survival of post-settlement larvae are variable, with occasional high mortalities (>90%) reported at around 3 weeks (L Tong, National Institute of Water and Atmospheric Research, pers. comm.) when paua are *c.* 700μm long and their radulae are developing more lateral teeth. High mortality may, therefore, be associated with a change in food requirements, the provision of which may depend on the mixture of available diatoms. Another factor may be oxygen depletion in the boundary layer on the surface where post-larvae grow (Searcy-Bernal 1996). Specially formulated artificial foods (*e.g.*, Knauer *et al.* 1996), or the provision of specific diatom cultures, may help reduce variability in survival and growth at this stage of development. A major programme funded by FoRST, attracting $182,000 annually (Anonymous 1996b), deals mostly with these early stages of post-larval survival and growth.

Despite the unknowns and the occasional problems with post-larval survival, it seems unlikely that this is a major impediment to the development of the paua farming industry at this stage. While variable early survival has a real cost, it is probably not great overall. Furthermore, hatcheries have been able to produce the numbers of juveniles required by industry so far. The problem of post-larval survival may become more important as industry expands and requires vast numbers of juveniles for year-round production. This will entail having an optimal mixture of food and conditions for all phases of juvenile growth, allowing predictable costings and cash flow in hatchery production.

Table 3 Current paua farming ventures in New Zealand (data supplied by R. Beattie, New Zealand Abalone Farmer's Association, October 1996).

A. Geographic spread

Region	Number of farms
Auckland/Northland	1
Taranaki	2
Canterbury	2
Otago	1
Stewart Island	2

B. Types of system used

Type	Number of farms
V-tanks	3
High-sided, narrow tanks	1
Stacked, flow-through	1
Barrel culture	1
Unknown/Undecided	2

C. Species used

Species	Number of farms
Haliotis iris	7
Haliotis australis	1
H. iris and *H. australis*	1

Food/diet/nutrition requirements

Paua are weaned from diatoms onto macroalgae at around 5mm shell length. In terms of natural food, a mixed diet of several species of brown and red seaweeds seems to produce the best growth of *Haliotis iris* (Wilson 1987, Stuart and Brown 1994). Artificial foods made in New Zealand and elsewhere are available and are used in at least two industry facilities. One major problem with food and nutrition in New Zealand, however, is that there are few published and, therefore, appraisable research results on the effects of different diets on long term growth of juveniles to marketable sizes. There is no published research on the nutritional requirements of paua. Furthermore, provision of the large quantities of natural seaweeds necessary for a commercial facility requires an algal harvesting permit, few of which have been granted so far.

Food is a major cost in any production facility. Artificial food may currently be too expensive for economic viability (Fleming *et al.* 1996) and the costs of gathering, transporting and feeding natural seaweeds on a commercial scale are largely unknown.

Husbandry/tanks/systems

Several systems of rearing paua are used in New Zealand. The V-tanks advocated in instructional courses (Tong and Moss 1992, Tong *et al.* 1992) have the advantages of a large surface area, easy access for feeding and cleaning, and availability. One disadvantage is that sorting and removing paua can be back-breaking work. Vertically stacked, round tanks are planned for at least one facility, while at least one other uses narrow, high, rectangular tanks. Coupled with tank design is the type of water system used. In southern New Zealand, for example, where water temperatures range from around 7 – 17°C, there may be advantages in heating water during the coldest winter months. Unpublished research indicates that paua feeding is severely reduced below 10°C, whereas paua will continue to feed and grow when the temperature is maintained above 15°C (Schiel unpublished, *c.f.*, Nie *et al.* 1996). Each of these systems has its own characteristics of water flow, air supply, and methods of feeding and handling paua.

Regulatory procedures

New Zealand has many regulations relevant to the aquaculture industry. These involve local, regional and national considerations, especially in compliance with the Resource Management Act (1991) which requires impact assessments for commercial development of land and use of natural resources. In a land-based facility, permits are required for land use (*i.e.*, compliance with local and regional plans), water intake and water discharge. The effects of the discharge need to be detailed, and modification and on-going monitoring may be required. A freshwater farming permit is required to hold animals for land-based aquaculture, and a marine farm license is needed for sea-based operations (such as barrel farming; *c.f.*, Aviles and Shepherd 1996). To buy and sell paua, a fish receiver license is required. If natural seaweeds are used as food, an algal harvesting permit must be held.

The costs in time and money in obtaining all of the necessary permits can be high, especially if there are objections and proceedings are drawn out. While the procedures may be expensive and administratively cumbersome, the regulatory requirements by themselves are probably not an impediment to industry progress at this stage.

Marketing/product development

In New Zealand at least, it is not yet clear what the market is for cultured paua. There has been much talk about "cocktail-sized" paua (around 70mm shell length) but no clear markets have yet been established. There is also considerable interest in paua "pearls", mostly blister pearls (or mabe) grown on the inside of shells. To date, however, the incipient industry has not been market-driven, and many argue that this has been the major reason for such slow progress in the development of the paua farming industry.

Investment

Potential investors have been wary about aquaculture since the late 1980s, when it became clear that the hype surrounding the industry was not producing quick results. There are high start-up costs in land, plant, materials, and permits, and there is no clearly established market for mass-produced small paua. There is a long lead time to produce even moderate cash flow, and a longer time for real profits. No single system of plant, feeding and techniques is readily available to give a reasonable degree of predictability about production and costs. Many of these problems have not been resolved sufficiently to attract investors.

In many ways, there are striking parallels between the paua culture industry today and the mussel (*Perna canaliculus*) industry in its early stages of development in the 1970s. Most investors are individuals or small companies, often beginning with inadequate longer term finance. As in the early mussel industry (Meredyth-Young and Waugh 1985) many types of production systems are used and markets are not clearly defined. It was not until more standardized equipment and procedures were developed, coupled with the involvement of larger fishing companies in terms of finance, processing plants and marketing, that the mussel industry underwent major expansion. Major steps in progress were the development of specialized spat collecting methods and the evolution and availability of separate growing and harvesting specialties, so that one operator no longer had to be responsible for the entire process. Some degree of standardization and specialization will certainly be necessary in the paua industry, but it is too early to see exactly what form this will take in New Zealand.

Paua farms in New Zealand

Most paua farming ventures in New Zealand are in an early stage of development. The geographic spread encompasses the length of the country from Auckland in the north to Stewart Island in the far south (Table 3A). Most farms are in the cooler waters from Taranaki (west coast, North Island) south. The most common system being used is the V-tank, although there is a wide range of types, with two operations still in the early planning stages (Table 3B). Seven farms will use *Haliotis iris*, one *H. australis*, and one both species (Table 3C). The products will be cocktail-sized paua and mabe pearls.

Husbandry and system interactions

Several models of husbandry and systems are being developed throughout the country. This diversity may be necessary because of regional differences in water temperatures, particularly with the significantly warmer water in northern New Zealand (monthly averages between c. 12 and 21°C) and site-specific differences in water quality and access, even within regions. There is considerable debate about which combination of traits, species and systems is best suited to any given operation. There is also the suspicion that in many cases the sites for paua farming are chosen more for convenience or prior land ownership, rather than for the quality of the site for paua farming.

In southern New Zealand, heating the water during the coldest winter months could result in increased growth of meat and shell, although there is no published information to gauge this relative to costs. If water heating is used, heat pumps will be required and a re-circulating water system that provides constant temperatures to all tanks will be necessary. Also needed will be a filtration system to remove major wastes and a bio-filter system to maintain water quality. Such an integrated system has yet to be tested on a commercial scale. It will also require the use of food that will not clog filters; the break-down of artificial foods can cause problems with this. Size-specific stocking densities will have to be worked out, and the entire system will have to permit easy removal of faeces, food wastes, and paua. Few of these have been subjected to direct research in New Zealand.

An ancillary problem relating to paua aquaculture is the provision of juvenile stock. It seems clear that specialized hatcheries will need to be developed to provide small paua (5–10mm) to other operations. The current price (October 1996) for juveniles ranges from 1.4 to 4 cents per mm, but it is felt that for profitability in an on-growth facility, NZ$0.02 per mm may be the maximum affordable for commercial viability (R. Beattie, NZ Abalone Farmer's Association, pers. comm.).

One problem at this stage is that there is not enough volume of production to keep the price of juveniles low, and with new operators being hesitant to buy paua at a higher price, the demand for juveniles is not steady or high enough to produce economy of scale.

The problem of stock provision is more problematic if pearls are to be produced. The minimum implant size of paua will be *c.*75mm, or 2–3 years old. With the minimum legal size for the natural fishery at 125mm, it is not clear where the volume of appropriately sized paua will come from for a commercial venture in paua pearls.

Provision of food

The nutritional requirements of paua are largely unknown and the quality and use of particular foods will be influenced by the paua species, temperature, production of wastes and the stocking density of paua in particular systems. The provision of quality food that is also economically viable is an on-going concern in New Zealand, as it is elsewhere (Fleming *et al.* 1996). For artificial foods, the best formulation for fast growth, efficient conversion, meat quality, taste and nacre production is still being developed.

The amount of food required by a commercial-scale facility will depend on stocking densities, sizes of paua, water temperature and duration of feeding by paua. "Daily intake" expressed as % Body Weight consumed per day averages *c.* 4% for 7–20mm abalone at 18–22°C and *c.* 1.1% for 25–50mm abalone (from Table 6, Fleming *et al.* 1996). However, there may be considerable wastage from uneaten food and decomposition, and the overall amount of food added to a tank (the "ration") to maximize growth rate may be double the daily intake value (Uki *et al.* 1985, quoted in Fleming *et al.* 1996). With the cost of artificial food presently high (an average of *c.*NZ$4.50 per kg; Table 1, Fleming *et al.* 1996), the economics of meat production of paua are currently unsatisfactory. Of course, this may change with continued improvements in growth rates, conversion ratios, reduced wastes, and reduced food costs, but there is clearly a long way to go for demonstrable economic viability using artificial food alone.

If natural seaweeds are to be used it is not certain which species are best, if permits to harvest them will be granted, and how much will be needed. A commercial facility clearly will require hundreds of tonnes per year, but the source of suitable seaweeds relative to the location of farms, the costs of collection and transport are mostly unknown, yet crucial to the planning necessary in a commercial operation.

Research scale and experimental designs

If research is to aid the development of industry, the science will have to be of a high standard and at a scale appropriate for transferral to commercial use. Searcy-Bernal (1994) presented a timely reminder that not only are rigorous experimental designs testing specific hypotheses necessary in aquaculture, but also that some measure of the power of a test to detect an effect should be determined before non-significant results are interpreted. He illustrated his points by citing an example from his own research (Searcy-Bernal *et al.* 1992) in which the effects of benthic diatoms, abalone mucus and GABA on settlement and survival of larvae were tested. Using Analysis of Variance a significant effect was detected at settlement, but the difference among treatments was not significant after 22 days, even though GABA produced an average of 2.6 times increase in survival over the least effective treatment (diatoms). Two major points are germane: that the statistical power of the test to detect an effect was only 19%, and that accepting the null hypothesis of no treatment effect would discourage the use of GABA, even though it might produce significant improvements in commercial operations. About a third of the papers in Searcy-Bernal's (1994) review interpreted a non-significant null hypothesis as if it were true, and no papers reported the statistical power.

Much research in aquaculture is done on relatively small scales over short time periods. However, this may not be particularly useful to an industry with large facilities within which paua need to be grown for two to three years. An illustrative example from New Zealand research is a feeding study comparing growth of small paua (15 – 25mm) on four algal diets (Stuart and Brown 1994). A

recirculating system with a total volume of 250 litres was used, with 5.2 litre glass jars used to hold treatments. There were four algal treatments, each containing three replicate jars with 40 paua. The experiment measured growth rates over 8 weeks when the water temperature was 15°C. *Gracilaria* produced growth rates of 9.7 ± 6.1 mg per day ($\bar{x}$ ± SE), *Macrocystis* 5.9 ± 3.3, *Ulva* 0.8 ± 2.1, and a mixed algal diet 11.6 ± 8.1. Using Covariance Analysis (comparisons of the different diets over time), they concluded that *Gracilaria* and mixed algae produced significantly greater growth rates than the other two treatments. Three points are relevant in applying this type of experiment to commercial use. 1) The experimental scale is so small that results may not translate to the much larger systems used in commercial culture. The spacing of paua, aeration, water flow, and effects of wastes are likely to interact in a different way in a large system. 2) No F-values were given in the experiment (only p-values were cited) and the variation due to separate jars within treatments was not separated from individual paua variation with treatments. The assumption is, therefore, that there was no between-jar within-treatment variation. These omissions combine to make it difficult to assess the power of the test used to detect effects. Because the individual variation within treatments was so high (standard deviations ranged from 22.9 to 88.4) and there were relatively small differences among three of the treatments, the power of the test to detect effects was probably low. In this case, this did not lead to a Type II error because the result was significant. 3) The treatments lasted for only eight weeks. Because of this short time span, compounded with the great variation in growth within treatments, the results may not be particularly helpful for a commercial operation.

A recurring lesson is that aquaculture researchers must continually grapple with high within-treatment variability among individual paua combined with relatively small effect sizes. There are usually logistic constraints on the number of treatments and replication possible, all of which combine to yield generally low statistical power in the critical tests. This may be especially serious where percent survival is the variable being measured. In this case, there is no within-replicate variation (as is the case where several paua are measured for growth within replicate containers) and, therefore, there is a need for greater replication of containers within treatments to achieve a reasonable level of statistical power.

The role of the market

Because the aim of aquaculture is to make a profit, the combination of production system traits, growth of paua, and relevant research will affect the ability to attract investors. Not only will commercial facilities be increasingly driven by market forces, centred on identifiable and branded products, but there will be increasing pressure on scientists to interact with industry to solve problems. A major challenge to scientists, at least in New Zealand, is to demonstrate the relevance of research and science to the aquaculture industry through high quality research that leads to real commercial applications.

What can research accomplish?

I see the major categories needing research attention for the advancement of paua culture in New Zealand as: overall system development and integration, including the interaction with foods; food development both for recently-settled and later stages of paua to achieve better survival, greater growth rates and lower variability; algal harvesting effects and techniques, to provide the information necessary for more permits to be granted and for gauging the relative costs and benefits of artificial vs natural foods; and perhaps a better understanding of the genetics affecting meat quality and colour, growth rates, and shell colour.

Overall, despite the current progress of the paua culture industry in New Zealand, there is good reason to be cautious in optimism. Many places worldwide are further developed in abalone aquaculture, and wild stocks of paua are still reasonably plentiful, so there will have to be very good marketing to sell products and keep prices up to meet the expectations of investors. Some successful niche markets are likely to arise, and progress in the production and marketing of paua pearls may

prove to be the greatest success. However, history to date encourages me to wait and see, rather than to predict a clear road ahead.

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Growth and demography of paua *Haliotis iris* (Mollusca: Gastropoda) in northeastern New Zealand

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Keywords: paua, abalone, *Haliotis iris*, growth, recruitment, population structure, New Zealand.

Abstract

The growth and demography of an unfished population of paua (*Haliotis iris*) was examined within a marine reserve on the northeastern coast of New Zealand from 1986 to 1991. Size-frequency analysis showed that recruitment of paua was regular and annual. Paua were found and measured in the field down to 4 mm shell length and juvenile paua were individually tagged down to 8 mm shell length. Growth estimates from tag-recapture and size-frequency analysis revealed that paua grew rapidly to a size of at least 70 mm shell length in approximately 3 years. Growth then slowed abruptly with negligible growth beyond 80 mm. In contrast to studies in other areas, paua rarely reached the legal takeable size of 125 mm. Tag-recapture studies revealed large variability in growth of the juvenile paua. The differences in growth and demography between southern and northern populations of paua has important implications for the future management of northern paua populations.

Introduction

Abalone (*Haliotis* spp.) form important commercial fisheries in temperate coastal waters around the world (Mottet, 1978). Good management of these fisheries requires a sound understanding of the biology and demography of the species.

In New Zealand, there are three species of abalone which are known under the collective name of paua (see Poore, 1969 for a review of the biology of these species). *Haliotis iris* Gmelin 1791, (the common or black-foot paua) is the largest, most abundant, and most commercially sought after. Most research on *Haliotis iris* has been concentrated in southern localities of New Zealand (Poore, 1969; 1972a; b; c; 1973; Sainsbury, 1982a; b; McShane *et al.*, 1988; Schiel and Breen, 1991; McShane *et al.*, 1994a; McShane *et al.*, 1994b; McShane and Naylor, 1995a; McShane and Naylor, 1995b). Although the northern New Zealand commercial paua fishery accounts for a small proportion of the total annual catch, recreational and traditional harvesting of paua in northern New Zealand is still extremely important. Apart from a preliminary survey by Dickson (1987) and a description of the reproductive biology of a northern population (Hooker and Creese, 1995), there have been no published studies from populations north of Coromandel. Although the McShane *et al.* (1994a) study on 61 populations of *H. iris* covers a wide geographic range, it does not include sites north of the Coromandel Peninsula. Many previous studies on gastropods have shown large spatial variability in population size structures, growth rates, recruitment and survival patterns (Underwood, 1979; Day and Fleming, 1992; McShane *et al.*, 1995a; McShane *et al.*, 1995b). It is therefore likely that demographic information from southern populations of *Haliotis iris* in New Zealand may not apply to northern populations. Densities of *Haliotis iris* in the most suitable habitats within the Leigh Marine Reserve (which has been totally protected since 1976 and might therefore be expected to

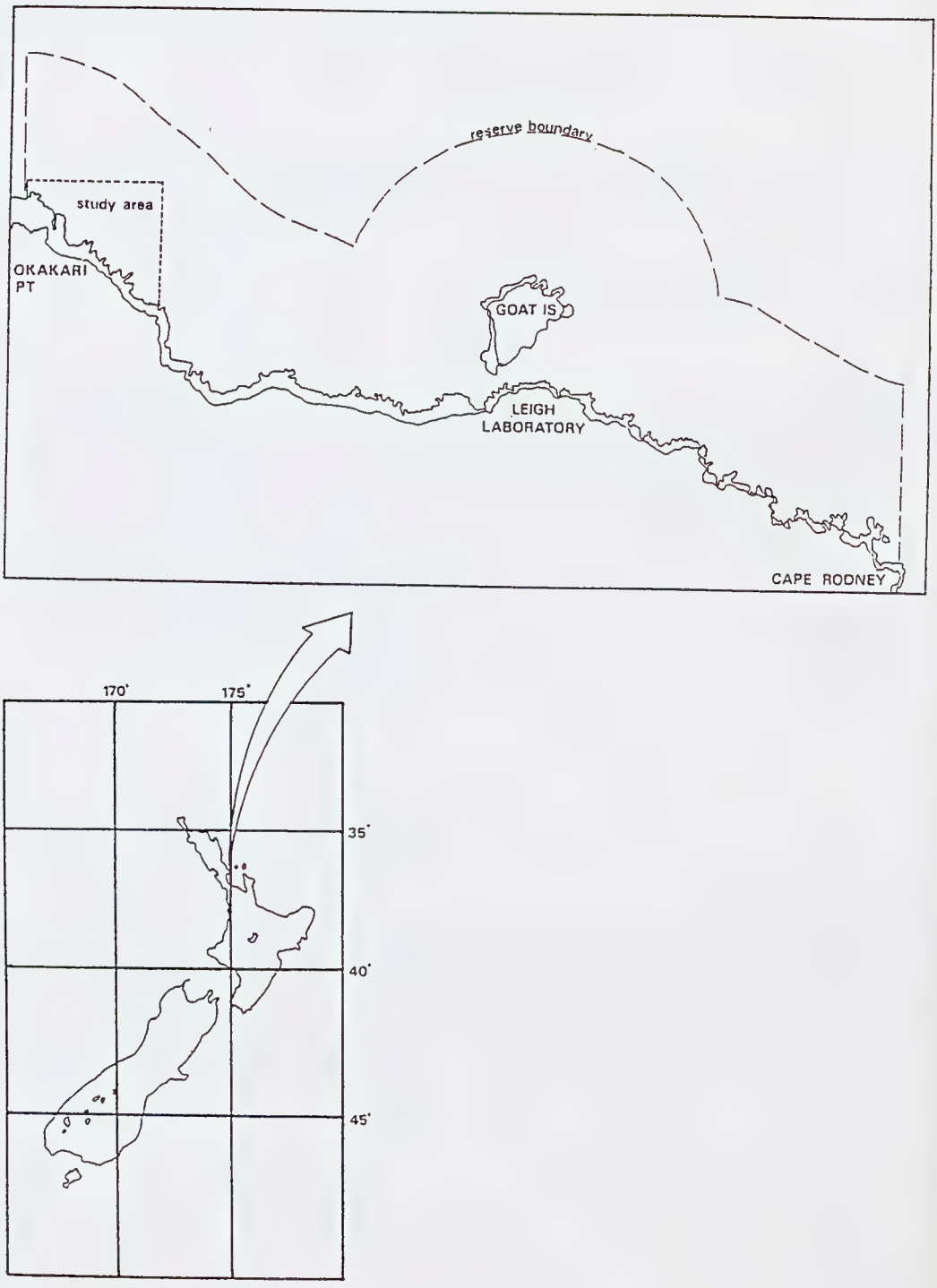

Figure 1 Map of the North Island of New Zealand showing the location of the study area at Okakari Point in the Leigh Marine Reserve and the nearby University of Auckland's Marine Laboratory.

have near natural population sizes) range from 6 to 14 m^{-2} (Hooker and Jeffs, unpubl. data). This compares with densities of 16 to 60 m^{-2} reported by Sainsbury (1982a) for southern populations. *Haliotis iris* in the Leigh Marine Reserve rarely reach 110 mm in length, lower than the minimum legal size of 125 mm and well below sizes of 160–170 mm commonly reached by paua from the South Island (Poore, 1969; Sainsbury, 1982a).

This paper describes a study of an unfished population of *H. iris* over six years (1986–1991) in northeastern New Zealand. The aim of this study was to examine and quantify habitat utilisation, temporal changes in population size structure and growth, and to link previously published information on the reproductive cycle of northern paua (Hooker and Creese, 1995) with the observed recruitment of juveniles into the population. Finally, we make larger spatial scale comparisons of our work to previous work on *H. iris* populations from southern localities.

Methods

The research was carried out in the Cape Rodney to Okakari Point Marine Reserve near Leigh (36°16'S, 174°48'E), hereafter referred to as the Leigh Marine Reserve (Fig. 1). The physical and biological characteristics of subtidal habitats in this area are described by Ayling (1976), Leum and Choat (1980), and Creese (1988).

Microhabitat and depth distribution: In November 1987, three sites (approximately 100 m apart) were selected at Okakari Point at the northern end of the Leigh Marine Reserve (Fig. 1). At each of the three sites a 60 m tape was laid out perpendicular to the shore, starting from the upper limit of the sublittoral fringe (*sensu* Morton and Miller, 1973). The transects were of sufficient length to extend from the sublittoral fringe through the "shallow broken rock" and into areas of "bare rock" habitat (*sensu* Ayling 1978). Paua are rarely found in any of the deeper habitat types (>10 m) within the reserve. A single quadrat (1 m^2) was sampled every second metre along the length of the transect while SCUBA diving. All paua in each quadrat were counted and measured *in situ* to the nearest millimetre using vernier callipers. The position of each paua was also designated to one of three microhabitats:

1. Cryptic: totally hidden under boulders so the paua could not be seen until the boulder was moved.
2. Partially Exposed: the paua could be seen without moving the boulder, but was partially hidden.
3. Exposed: the paua was totally out in the open and easily seen without moving any boulders.

Each transect was arbitrary divided into three twenty metre sections, which roughly corresponded to different depth strata. The first section (shallow) corresponded to 0–2 m depth below the sublittoral fringe. The second section (mid) corresponded to 2–4 m depth from the sublittoral fringe and the third section (deep) corresponded to 4–6 m. The substratum is extremely variable over this depth range, consisting of large flat sections of bed-rock with small caves and crevices, or large boulders and vertical bed-rock (2–5 m high) intermingled with smaller boulders and pebbles.

Morphometric relationships in paua: To test the assumption that shell length is an adequate measurement of animal size, a size range of adult paua were collected monthly (as described in Hooker and Creese, 1995) along with collections of smaller individuals. The shells of all animals were measured, and then each animal was removed from its shell, drained of excess water and weighed.

To assess whether wet weight measurements were an adequate description of weight, a sample of paua was dried in an oven at 78°C. After one week the animals were removed and re-weighed to the nearest 0.1 gram, and correlations obtained for the relationship between wet and dry weights.

Tagging: Adult paua were tagged using numbered plastic tags which were attached with stainless steel wire threaded though the most posterior respiratory pore. Paua could be reliably tagged down to a size of approximately 70 mm by this method, but in smaller paua the wire pulled through the thinner shell. A small number (29) of adult paua were tagged by using a small amount of a two-part

epoxy putty, which was pressed onto the shell near the apex, and a plastic tag was then embedded into the putty. Approximately 1300 adult paua were tagged between October 1986 and February 1987 at four sites within the Leigh Marine Reserve, two at Okakari Point, one at Te Rere Bay and one at Cape Rodney at the southern end of the Marine Reserve (Fig. 1). All paua to be tagged were carefully removed from the rock surface, taken to a nearby intertidal rock platform, tagged and measured, then replaced as soon as possible. Tagged adults were re-measured twice in 1987 (June and October), and a small number (16) were re-measured at one site in early 1990. The tagged animals were re-measured *in situ* where possible.

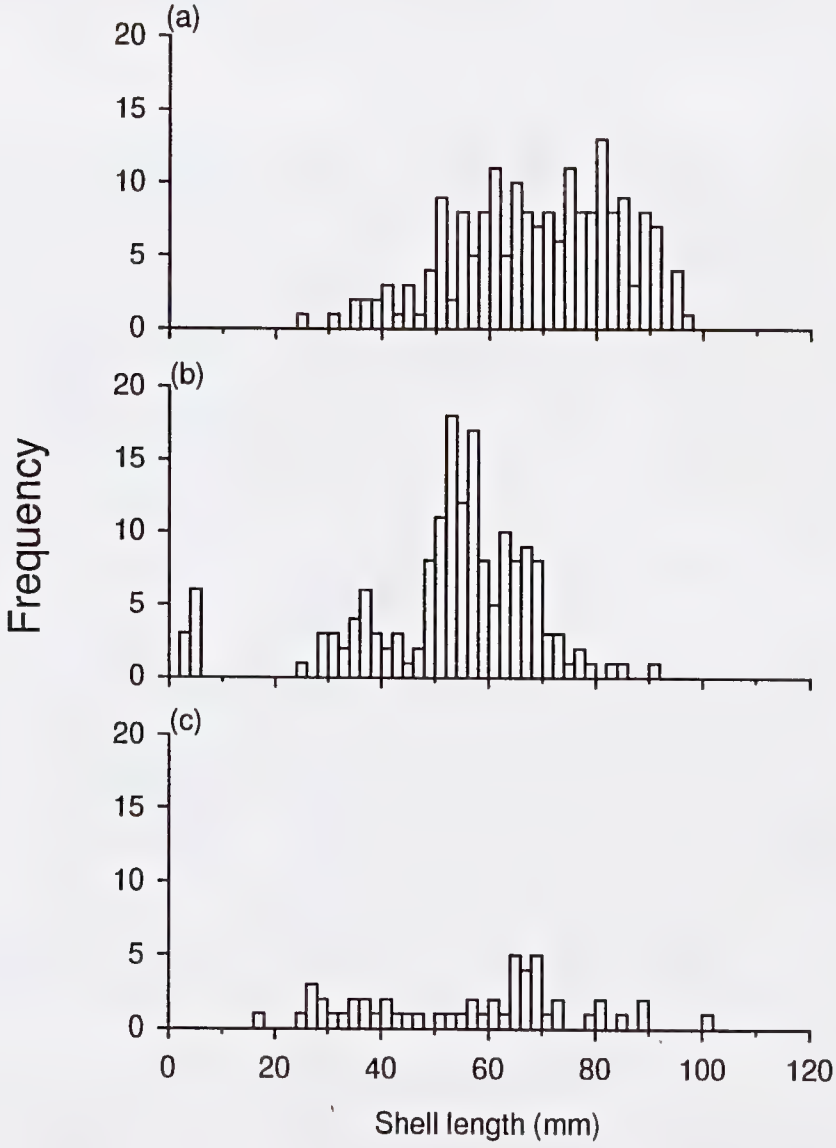

Figure 2 Length frequency distributions of the three transects combined and stratified with respect to distance from the shore. (a), shallow (0–20 m from sublittoral fringe) n=187. (b), mid depth (20–40 m from sublittoral fringe) n=166. (c), deep 40–60 m from sublittoral fringe) n=52.

Approximately 800 juveniles were tagged in the field at four sites at Okakari Point in February 1987. Tagged juveniles were returned to their original sites within a few minutes to minimise possible tagging effects. All juvenile paua (<70 mm) were tagged using numbered plastic tags (Hallprint), fixed to the clean exterior shell of the paua with cyanoacrylate-glue. A further sample of juveniles was tagged in May 1987, when the juvenile paua from the first tagging were also remeasured. All tagged juveniles were remeasured in October 1987. This was accomplished by methodically searching all boulders in the tagging site and the surrounding area.

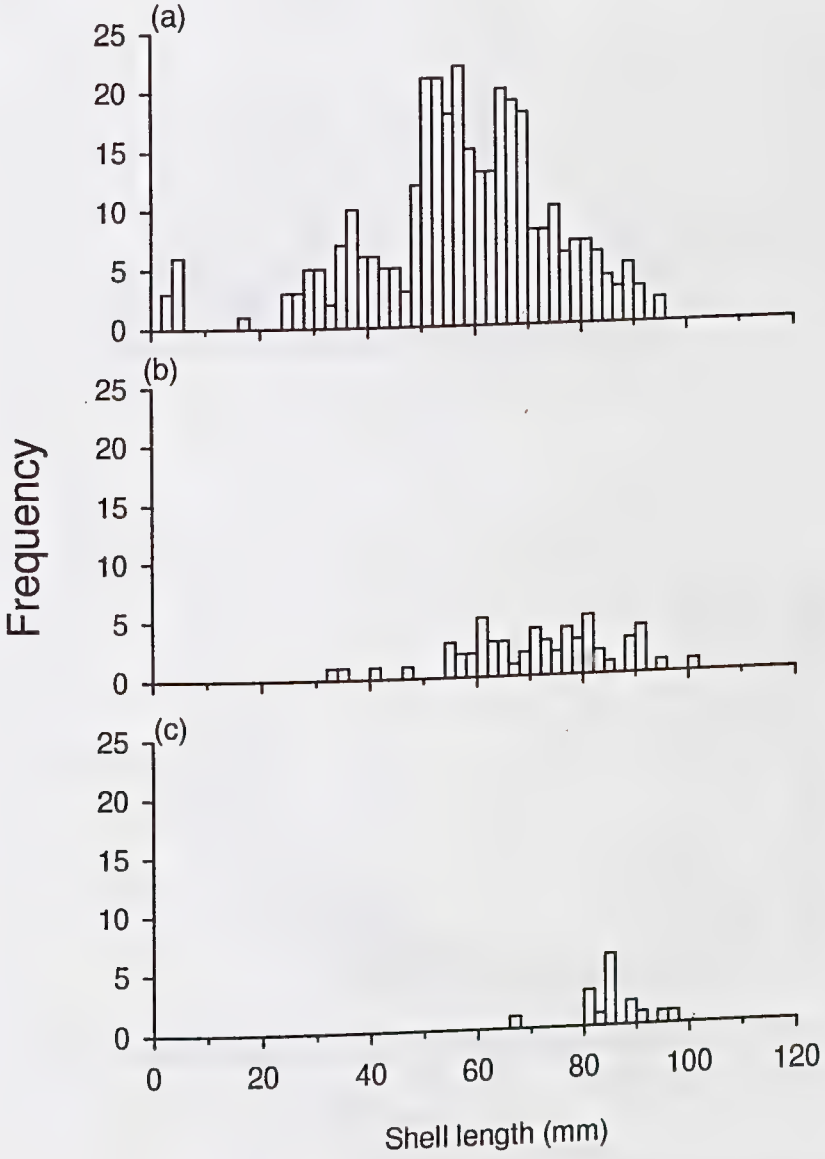

Figure 3 Length frequency distributions of the three transects combined and divided with respect to micro-habitat use. (a), "Cryptic" paua were totally hidden under boulders, n=331. (b), "semi-exposed" paua could be seen without moving the boulder but were partially hidden, n=58. (c) "exposed" paua were totally out in the open, n=16.

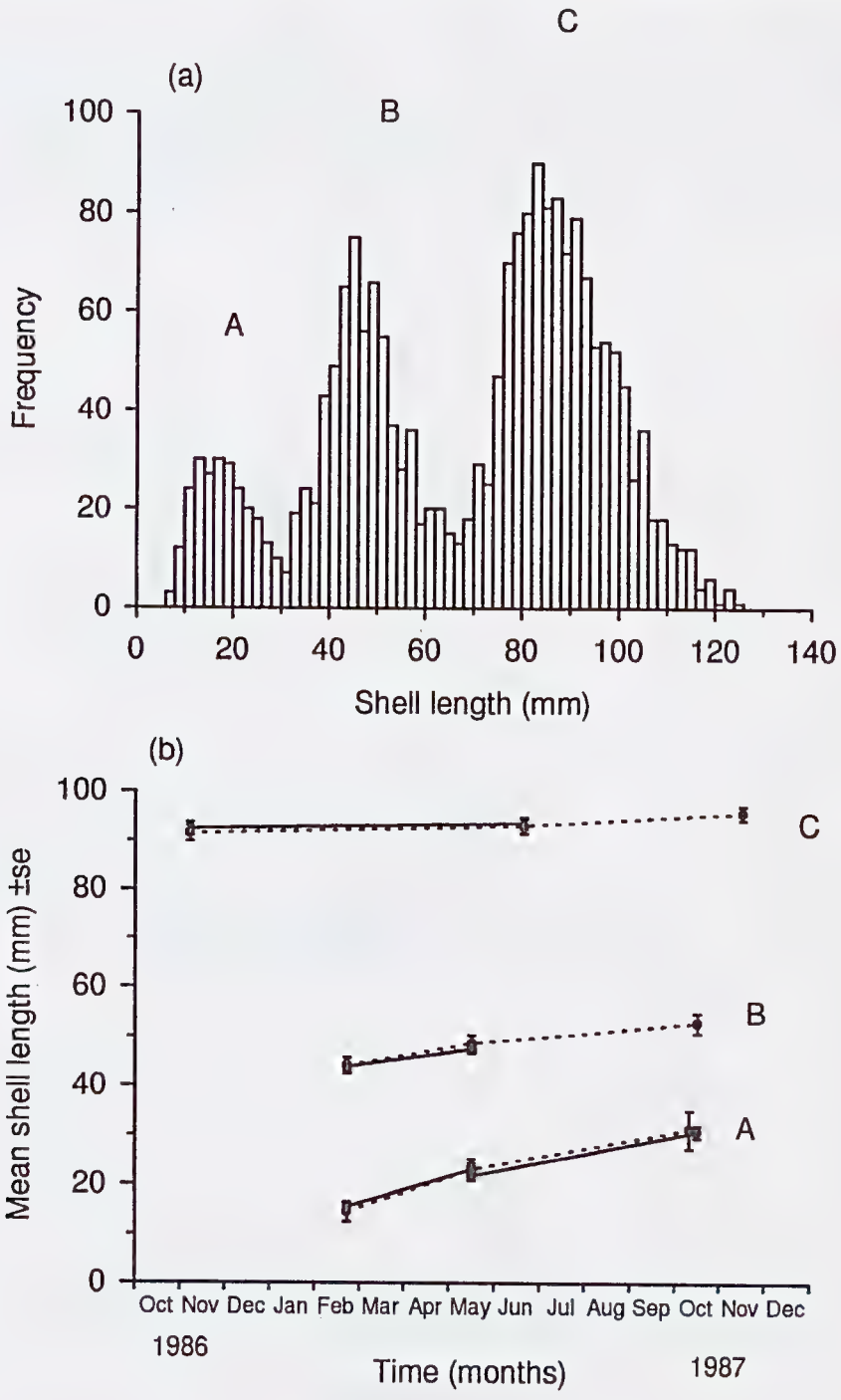

Figure 4 (a) Length frequency distribution of the initial lengths of all tagged paua from the Leigh Marine Reserve. (b) Growth of the mean size of each of the three tagged cohorts from (a). The dashed line represents paua that were recaptured twice.

Population sampling: Paua were measured in the boulder habitat at Okakari Point on 12 occasions between February 1986 and February 1991. A 1 m^2 square quadrat was thrown haphazardly into areas of boulders. Each quadrat was methodically searched by turning over all moveable boulders. All paua found were measured and replaced onto the boulders. This procedure was repeated until at least 200 paua had been measured. Cohorts were identified and their mean lengths and standard deviations calculated using polymodal size-frequency analysis (ELEFAN; Pauly and David, 1980; Pauly and Ingles, 1981).

Results

Microhabitat and depth distribution: There were only slight differences in population structure on the three transects. New recruits were found only on the third transect. Mean densities (± standard error) were 6.1 ± 2.2. m^{-2} for transect one, 2.6 ± 0.8. m^{-2} for transect two and 4.5 ± 1.8. m^{-2} for transect three. Paua were less abundant further from shore (Fig. 2), in slightly deeper water, where there was less boulder habitat with more areas of flat bed-rock and sand. Although the full size range of paua was found at all depths, there was a tendency for more larger animals in shallow water. When the transects were pooled across the three micro-habitats (Fig. 3), paua of all sizes were mostly cryptic (81.7%). Very few paua (only 4%) were found totally out in the open, and all but one of these exposed individuals were over 80 mm in length.

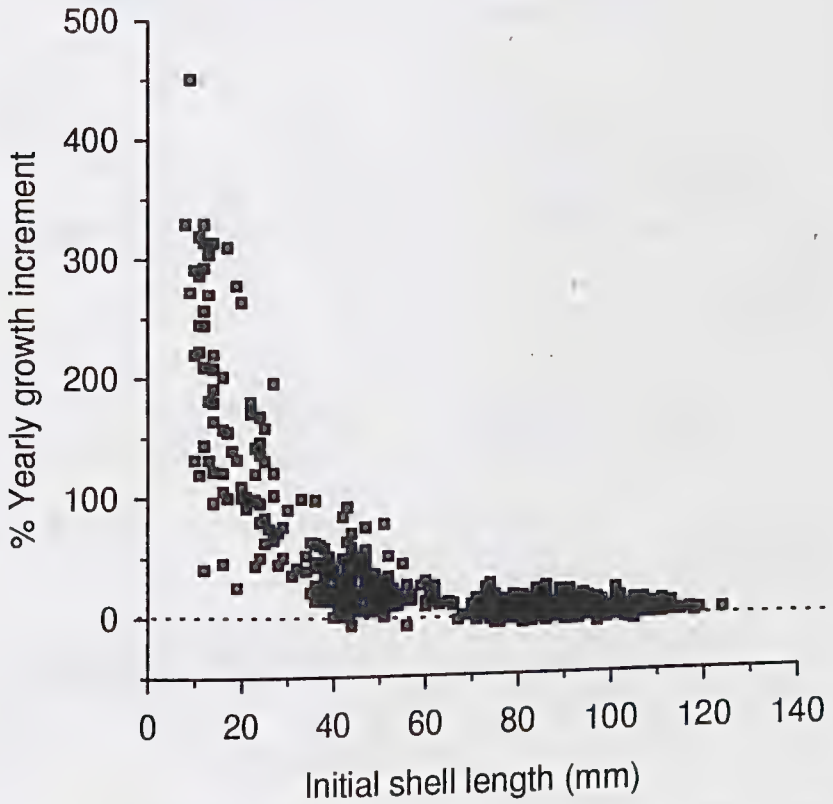

Figure 5 Plot of the percentage growth increment, standardised to one year's growth, versus the initial length of all tagged paua. Growth increments below zero are measurement errors that have been multiplied to a year's growth.

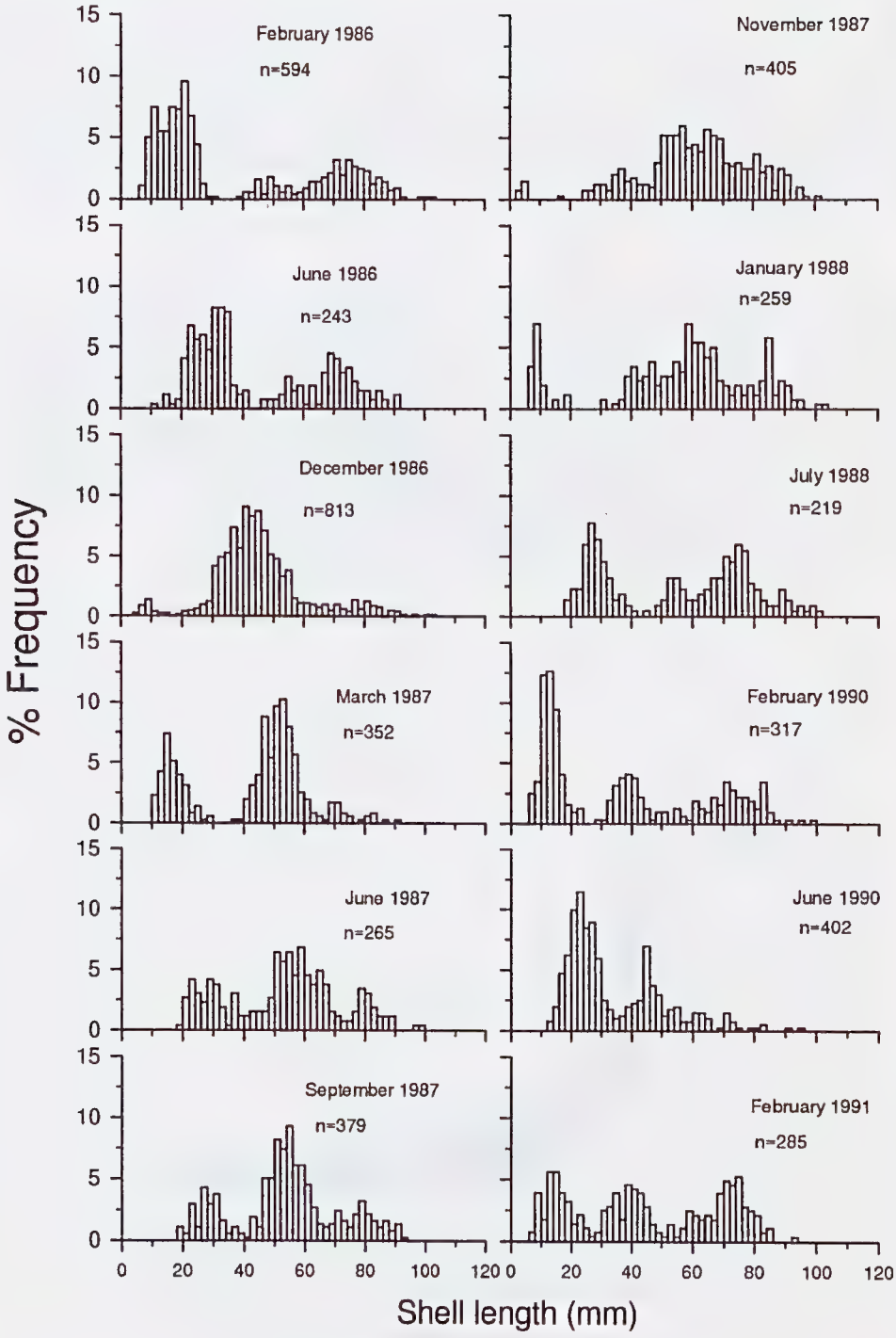

Figure 6 Length frequency histograms of juvenile paua at Okakari Point from February 1986 to February 1991. Samples were collected from within the boulder habitat where there are mainly juvenile paua and therefore underestimate the adult fraction in the population.

Morphometric relationships: The correlation between wet weight and dry weight of paua over the whole range of sizes had an r^2 value of 0.998, indicating that wet weight was an adequate estimator of weight. The shell length to body weight relationship throughout the size range was calculated as $W=0.0000236\ L^{3.29}$ (W=wet body weight in g; L = shell length in mm).

Tag-recapture: The initial lengths of tagged paua fell into three distinct cohorts (Fig. 4a) which were representative of the paua population at Okakari Point at that time. The recapture of tagged paua was not biased to any particular size group with a similar proportion of each of the three cohorts being subsequently recovered. An estimate of the growth rate of the three groups of tagged paua was obtained by plotting the mean length of each cohort against time (Fig. 4b). Assuming that spawning occurred around August/September (Hooker and Creese, 1995; see also Fig. 7), it is clear that the first mode in Fig. 4a consists of 0+ animals and hence that newly settled paua grew to approximately 30 mm in their first year and to approximately 52 mm in their second year. The slope of the line changes between sampling periods (Fig. 4b) suggesting seasonal variations in growth with a smaller mean increase in length over the winter months.

Growth of smaller tagged paua was highly variable (Fig. 5). For individuals less than 25 mm, the annual increase in length varied from 450% to under 50%. Increments in shell length were smaller and much less variable for paua larger than 25 mm. The annual growth increment approached zero at around 75 mm in length and growth beyond this size was very slow.

Population sampling: The twelve samples collected from Okakari Point from February 1986 to February 1991 show discrete modal size-classes, suggesting strong pulses of recruitment between August and October each year (Fig. 6). Juveniles grew rapidly from the size at which they were first detected to merge with the adult mode after approximately three years. The mean size of the largest cohort stayed relatively constant through time. Because sampling was restricted to the boulder habitat, only the smaller cohorts were adequately represented; paua over 80 mm were mostly found outside this habitat. The ELEFAN software package fits normal curves to polymodal frequency distributions. In all but one case (the first sample in February 1986) the fitted curves did not differ significantly from the actual data sets (c^2 goodness-of-fit tests, $P<0.05$, d.f. ranging from 13 to 24). The mean sizes of the cohorts of juvenile paua, as determined by the ELEFAN analysis, are plotted on a linear time scale in Fig. 7. Estimates of growth of larger animals were not possible as the older cohorts coalesced above approximately 70 mm.

From 1986 to 1991 paua grew from 31 to 35 mm in their first year, and reached 55 to 60 mm in their second year (Fig. 7). Separation of the 2+ cohort was not always possible as cohorts had either coalesced or the information could not be reliably derived from the data. However, the general trend was for the cohorts to reach at least 70 mm by the end of their third year in the boulder field (Fig. 7). A possible seasonal component to the growth of paua in this sub-population was apparent over the winter (June to September) of 1987 when growth of all cohorts slowed markedly (Fig. 7).

Similar estimates of growth were obtained from the analyses of the size-frequency data and the tag-recapture data during the period February to October 1987. The mean size of tagged paua in February 1987 was 14 mm (Fig. 4b) and the estimated size of the corresponding cohort in the population size-frequency analysis was the same (Fig. 7). The tagging data revealed that the cohort had grown to a mean size of 23 mm by May 1987 and estimates from the size frequency analysis for this month also estimated the cohort at approximately 23 mm. By October 1987 the tagging data showed that the mean size of the cohort was 31 mm (Fig. 4b) and the size frequency analysis again gave the same result (Fig 7). This strongly suggests that tagging had a negligible effect on the growth rate of paua and that polymodal size frequency analysis was an accurate technique for assessing growth in this sub-population.

Discussion

Several haliotid species are known to show depth-related patterns (Poore, 1972c; Shepherd, 1973;

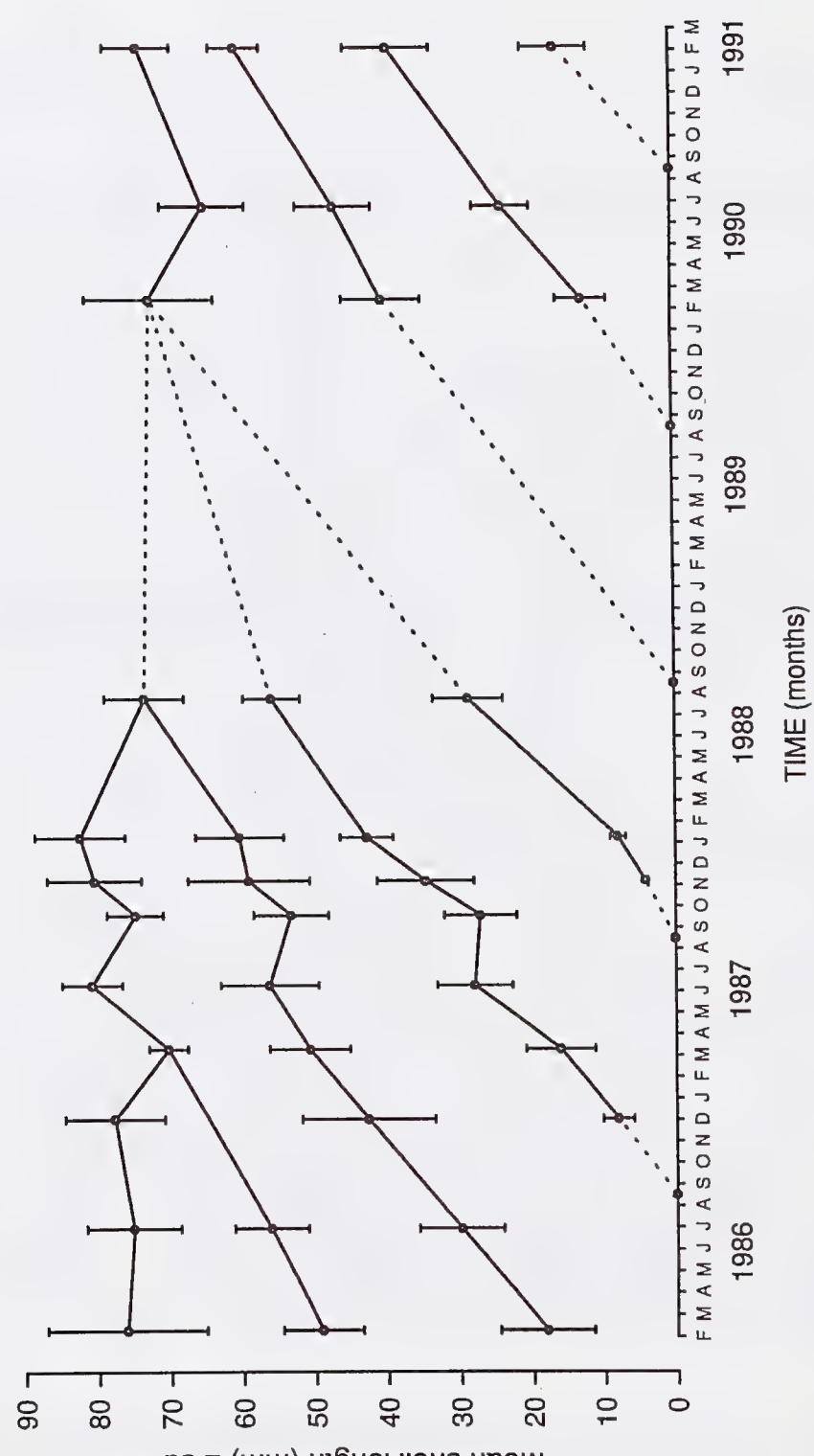

Figure 7 Mean length of cohorts, on a linear time scale, derived from length frequency histograms from February 1986 to February 1991 (Fig. 7). Error bars are standard deviation. Dashed lines represent areas of uncertain growth of cohorts and lines extrapolated back to the probable time of settlement (spawning) estimated from reproductive studies (Hooker and Creese, 1995).

Witherspoon, 1975; Schiel, 1992). In this study there was not a consistent change in population structure with depth for *H. iris* in the Leigh Marine Reserve. Any difference in population structure is possibly an artefact of available habitat rather than determined by depth itself. Both juvenile and adult paua can be found across the depth range sampled if there is suitable habitat (Hooker and Jeffs, unpubl. data).

Spatial separation of age cohorts on the basis of microhabitat type has been shown to be important for abalone in southern Australia (Shepherd, 1973; Prince *et al.*, 1988; Witherspoon, 1975; Shepherd and Turner, 1985), and for *H. iris* in southern New Zealand where paua begin to emerge from their cryptic habitat at the onset of sexual maturity (Schiel, 1992; McShane and Naylor, 1995a). Paua within the Leigh Marine Reserve do not appear to emerge from the cryptic habitat at the onset of sexual maturity (50–60 mm SL, Hooker and Creese, 1995). Although, there was a tendency for larger paua to be more exposed than smaller animals, paua of all sizes were still found under boulders and very few paua were seen on exposed surfaces at these sites. This is different from the pattern found for other haliotid species and for *H. iris* in southern localities. This difference of depth distribution and micro-habitat utilisation between *H. iris* in the Leigh Marine Reserve and more southern localities in New Zealand may simply be due to the much greater densities of paua found in southern populations, thus limiting the cryptic habitat available to the larger paua.

The size-frequency distributions from Okakari Point show single annual recruitment pulses in the years 1985–1990. However, reproductive studies of paua at Leigh showed that multiple spawnings occur over the late winter to early spring period (July to September) (Hooker and Creese, 1995; Neill, 1997), suggesting that successful recruitment may result from only one particular spawning event or alternatively that multiple settlements coalesced very rapidly into a single year class (Day and Fleming, 1992). Recruitment was observed in December 1986, with the emergence of a 0+ cohort with a mean size of approximately 8 mm SL and in November 1987 with a mean size of approximately 4 mm SL. Spawning and settlement in *Haliotis iris* are separated by less than 14 days (Tong *et al.*, 1987; Moss and Tong, 1992), and newly settled juveniles grow to 3–4 mm SL in 1–2 months. The observed recruitment in November/December therefore most likely originated from the late winter to early spring spawning, *i.e.* when the seawater starts to warm up from the annual minimum in August/September.

Growth of haliotids is often summarised by fitting growth curves of the von Bertalanffy type (Forster, 1967; Newman, 1968; Poore, 1972c; Hayashi, 1980; Sainsbury, 1982a; Shepherd and Hearn, 1983; Keesing and Wells, 1989; Day and Fleming, 1992). While useful in predicting future growth trends (in fished populations for example) and assuming that the assumptions of these growth models have been met (something that is not always explicitly demonstrated), it is often difficult to assess whether the generated growth curves adequately describe the actual growth of the population because few raw data are presented in scientific publications. It may be possible, for instance, to over- or under-estimate early growth (Poore, 1972c) or to obtain a wide range of growth estimates from a given set of size data (Grant *et al.*, 1987; Prince *et al.*, 1988). Although our size-frequency data covered a reasonable time span (5 years), they came from a restricted habitat that did not include the full size range of paua, and there was also an 18 month gap in the time series. The tagging data covered a full size range of animals, but was restricted to a period of less than one year. Although the combination of these two data sets allowed us to confidently describe the growth patterns of paua in the Leigh Marine Reserve, we can see no merit in using them at this time to derive additional growth parameters for use in von Bertalanffy models.

Analysis of the tag-recapture and size-frequency analysis on raw data demonstrates that paua in the Leigh Marine Reserve grow to approximately 28–35 mm in their first year, 50–55 mm in their second year and 70–75 mm in their third year. Growth beyond 80 mm SL was negligible. This result is different from that obtained by Poore (1972c) and Sainsbury (1982a) for southern populations. Comparison of the growth rate of paua in the Leigh Marine Reserve population with that obtained by Poore (1972c) and Sainsbury (1982a) shows that paua at Leigh have an initially faster growth rate than southern paua to approximately three years old, but that growth slows abruptly after

approximately 80 mm. Southern paua have a slower initial growth rate but keep growing to a much larger maximum size than paua in this northern locality.

Paua in the Leigh Marine Reserve seldom reached sizes greater than 100–110 mm, with most adults averaging 70–90 mm. The largest tagged paua was 124 mm, and only two paua, of 126 mm each, were found over the legal size limit on the coast adjacent to the Leigh Marine Reserve. In southern paua populations, it is not uncommon for paua over 160 mm to be found, and Poore (1969) recorded an individual of 177 mm SL. The mean shell length from samples of adult *H. iris* from 61 populations from around New Zealand revealed that three out of the four populations in the northern half of the North Island were below 100 mm and all southern populations had a mean shell length over 100 mm (McShane *et al.,* 1994a)

Subtle small scale (hundreds of metres) ecological differences in abalone populations have been found to be important for their management (McShane and Naylor, 1995a). Marked differences in the ecology of *Haliotis iris* populations have been demonstrated here on a larger geographic scale (hundreds of kilometres). Northern paua populations have important demographic differences compared to southern populations. Their reproductive cycle is substantially different (Hooker and Creese, 1995), their habitat utilisation, densities and growth characteristics (as described here) are all different from southern populations. These differences will have important implications for the management and possibly aquaculture of this species in northern New Zealand.

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Isolation of a fungus from shell lesions of New Zealand abalone, *Haliotis iris* Martyn and *H. australis* Gmelin

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Abstract

Shell irregularities characterized by deposition of conchiolin on the inner shell surface of abalone, *Haliotis* spp., have been reported in populations along the South Island of New Zealand for over 50 years. A previously reported, but unidentified, fungus associated with these shell lesions is isolated for the first time from *Haliotis iris*, the black footed paua, and *H. australis*, the yellow footed paua, both obtained from Jacks Bay, South East Otago. Fungal hyphae measured 1.20 - 2.38 µm ($\bar{x}$ = 1.83 µm) in diameter. Occasionally, wider hyphae that measured 2.38 - 3.60 µm ($\bar{x}$ = 2.60 µm) in diameter with an apical swelling that measured 4.80 - 7.20 x 7.20 - 15.00 µm ($\bar{x}$ = 6.20 x 11.80 µm) were observed within lesions. Visible growth of aerial hyphae was observed on plates within 12 d at 16 °C on several media. Three morphologically indistinguishable isolates were obtained: two originated from *H. iris* shells and one from an *H. australis* shell. We examined 8 fungal and/or bacterial media and determined that the fungus grew best on a dilute medium (yeast extract, glucose, shell extract (+/-peptone agar) at 15 °C. Fungal growth was observed between 7 – 20 °C but not at room temperature (24 °C). Sporulation has not been induced, therefore the fungus is provisionally identified as a member of the Class Deuteromycotina.

No significant relationship between animal length and incidence of shell mycosis was observed. Abalone condition was not influenced by the shell mycosis in either species. The number of circulating haemocytes was significantly elevated in infected *H. australis* (1.19×10^7 cells ml^{-1}) relative to uninfected individuals (6.72×10^6 cells ml^{-1}) but not between infected and uninfected *H. iris*. No significant relationships were observed between the degree of fungal infection and protein levels of whole haemolymph, serum or sex (immature, male or female; $p<0.050$; N=34). We are currently attempting to determine the taxonomic placement of the fungus and further characterize its growth physiology and pathogenicity.

Introduction

Shell deformities characterized by the deposition of conchiolin on the dorsal region of the inner surface of the shell of abalone, *Haliotis* spp., have been reported in populations along the South Island of New Zealand for over 50 years (Sinclair 1963; Sainsbury 1977; Grindley unpublished data). Grindley (unpublished data) observed the presence of an unidentified fungus within shell lesions of affected abalone. Prior to our characterization, only two fungal species, *Haliphthoros milfordensis* Vishniac, 1958 and *Atkinsiella awabi* Kitancharoen, Nakamura, Wada and Hatai, 1994, have been described and/or isolated from abalone. Both of these fungal pathogens were associated with lesions in the soft tissues of abalone, *H. sieboldii* Reeve, 1846, from Japan. *Haliphthoros milfordensis* caused tubercle-like or flat lesions on the mantle, epipodium and dorsal surface of the

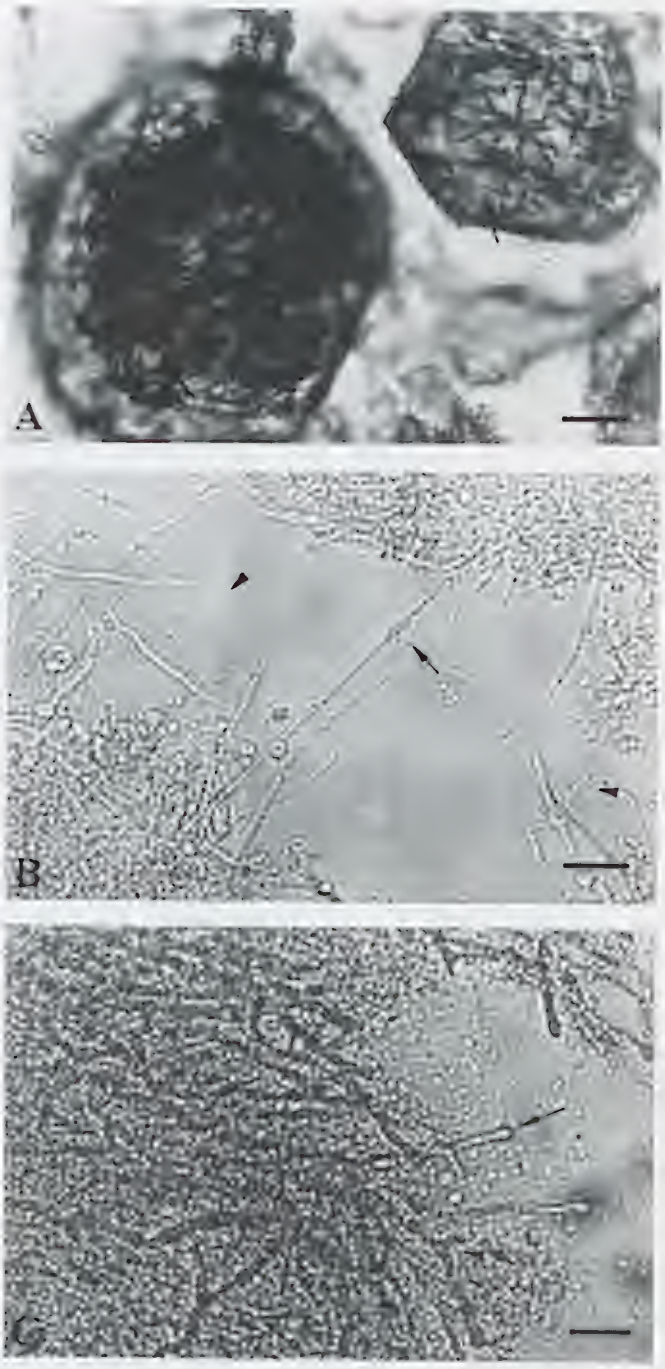

Figure 1 Lesion squashes. A. Polygonal conchiolin bodies from an advanced lesion penetrated with numerous fungal hyphae (arrows). Phase contrast. Bar = 22 μm. B. Wet mount of hyphae in a newly formed lesion. Note the septate nature of the fungus (arrow heads) and intercalary chlamydospore (arrow). Phase contrast. Bar = 16 μm. C. Wet mount of wider hyphae within a newly formed lesion . Arrow head denotes septa and a mycelial branch. Terminal swellings (arrow head) were frequently observed. Phase contrast. Bar = 15 μm.

foot (Hatai 1982). Clinical presentations of *A. awabi* resembled those of *H. milfordensis* and were described as raised tubercle-like lesions on the mantle and melanized lesions on the foot (Kitancharoen *et al.* 1994). Although marine fungi have been reported as invaders of calcareous materials such as barnacle tests and molluscan shells (e.g. *Pharcidia balani* (Winter) Bauch, 1936), no marine fungi have been identified or isolated from abalone shells (Kohlmeyer & Kohlmeyer 1979). We report the first isolation of a pathogenic fungus from shells of abalone from New Zealand. We isolated a fungus and characterized its growth characteristics. This study also assessed whether the shell mycosis affected the health of abalone as measured by a condition index and several hemolymph parameters.

Materials and methods

Abalone

Haliotis iris, the black footed paua, and *H. australis*, the yellow footed paua, were collected from intertidal and subtidal locations in Jack's Bay, Otago, New Zealand, on 3 November, 1995. Abalone were transported to the Portobello Marine Laboratory, University of Otago, and placed in two aquaria that received sand-filtered, full strength seawater at 13 °C. Abalone were fed *Macrocystis pyrifera* Bory, 1826, *ad libitum*. After tagging individuals, total weight (TW), shell weight (SW) and length (L) were determined prior to examination. A condition index (CI) was calculated for each animal: CI = {(TW-SW)/TW}. An aliquot of hemolymph was aseptically aspirated from the pallial sinus. The density of circulating haemocytes was quantified using a hemocytometer, and protein levels of whole haemolymph were determined according to the methods of Lowry (1951). In addition, an aliquot of whole hemolymph was centrifuged at 325 x g for 5 min, and the serum was analyzed for total protein as above. The shell of each abalone was removed and examined for lesions. Presence of lesions was quantified according to the following 0 to 3+ scale: 0) no lesion present; 1) lesion measured < 1 cm^2; 2) lesion measured 1–3 cm^2; 3) lesion measured > 3 cm^2. Tissues and shell adjacent to lesions were examined visually for penetration of fungal hyphae, and selected tissues were processed for routine paraffin histology.

Fungal Isolation

Shells with lesions characteristic of the abalone shell mycosis were surface sterilized with betadine and 70 % ethanol, followed by a rinse with 0.2 µm-filtered seawater. Small pieces of lesions were excised, placed on a glass slide and viewed by phase contrast microscopy (Figure 2). Soft lesions with visible hyphae were inoculated onto the following 4 media: 1) modified Vishniac medium (Shields 1990) supplemented with 100 ml l^{-1} of 10% (w/v) autoclaved (15 psi, 1 h) shell extract (MMV); 2) Glucose-yeast agar (Alderman & Polglase 1986) supplemented with 500 mg ml^{-1} Penicillin G, 500 mg ml^{-1} Streptomycin and 40 ml l^{-1} of 10% (w/v) shell extract (IM); 3) Sabauroud Dextrose Agar (Difco Laboratories, Detroit, Mich.) made with 50% seawater; 4) Marine agar (Difco Laboratories, Detroit, Mich.) supplemented with 40 ml l^{-1} of 10% (w/v) shell extract (MA). Plates were incubated at 15–16 °C under aerobic and microaerophilic conditions (using a candle jar) for up to 2 months.

Fungal Culture Conditions

To assess the best growth medium, colonies were subcultured in triplicate onto the following six media and incubated at 15 °C: MMV, IM, Brain Heart Infusion Agar (Difco Laboratories, Detroit, Mich.) with and without 2% added salt (BHI (+/-2% NaCl), Corn Meal Agar +2% NaCl (CMA, Difco Laboratories, Detroit, Mich.), and Potato Dextrose Agar +2% NaCl (PDA, Difco Laboratories, Detroit, Mich.). Colonies were also subcultured onto MMV, IM, BHI, and BHI+2% NaCl and incubated at 7, 15, 20 and 24 °C. Colony radius was measured to the nearest 0.1 mm every 1–3 wk to quantify relative growth rates at each of the four experimental temperatures. We attempted to induce

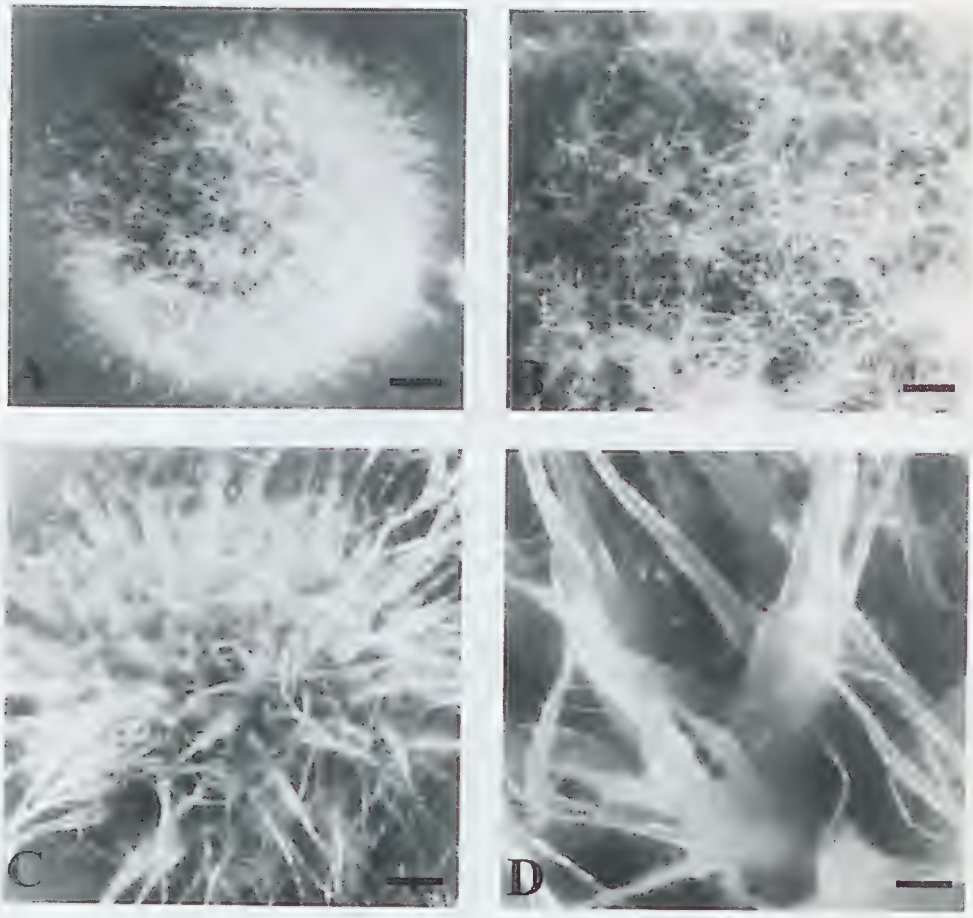

Figure 2 Two colony morphologies isolated in pure culture from abalone shell lesions. A. Colony with fine aerial hyphae. Bar = 540 µm. B. High magnification of fine aerial hyphae. Bar = 140 µm. C. Colony with stout erect thalli. Bar = 585 µm. D. High magnification of stout thalli. Note lateral fine hyphae. Bar = 150 µm.

sporulation using nutrient deprivation (colonies were inoculated into 0.22 µm-filtered seawater). Induction of sporulation was also attempted by incubating the fungus at 15 °C in the dark, and transferring them to near ultraviolet light (black light) for 10–12 hrs/day for 5–7 days each week (F. Dugan, American Tissue-Type Culture Collection, unpublished data).

Statistical Analysis

The Mann Whitney Rank Sum test was used to examine the difference in prevalence of infections between the two species examined. The Spearman Rank Order Correlation test was used to examine the relationship between abalone length, hemolymph parameters, sex and degree of shell mycosis. Differences in the average condition index of infected and uninfected individuals within a species and between the two abalone species used in this study was examined using a students t-test. The Pearson Product Moment Correlation test was used to examine the relationship between the degree of shell mycosis and an abalones' condition index.

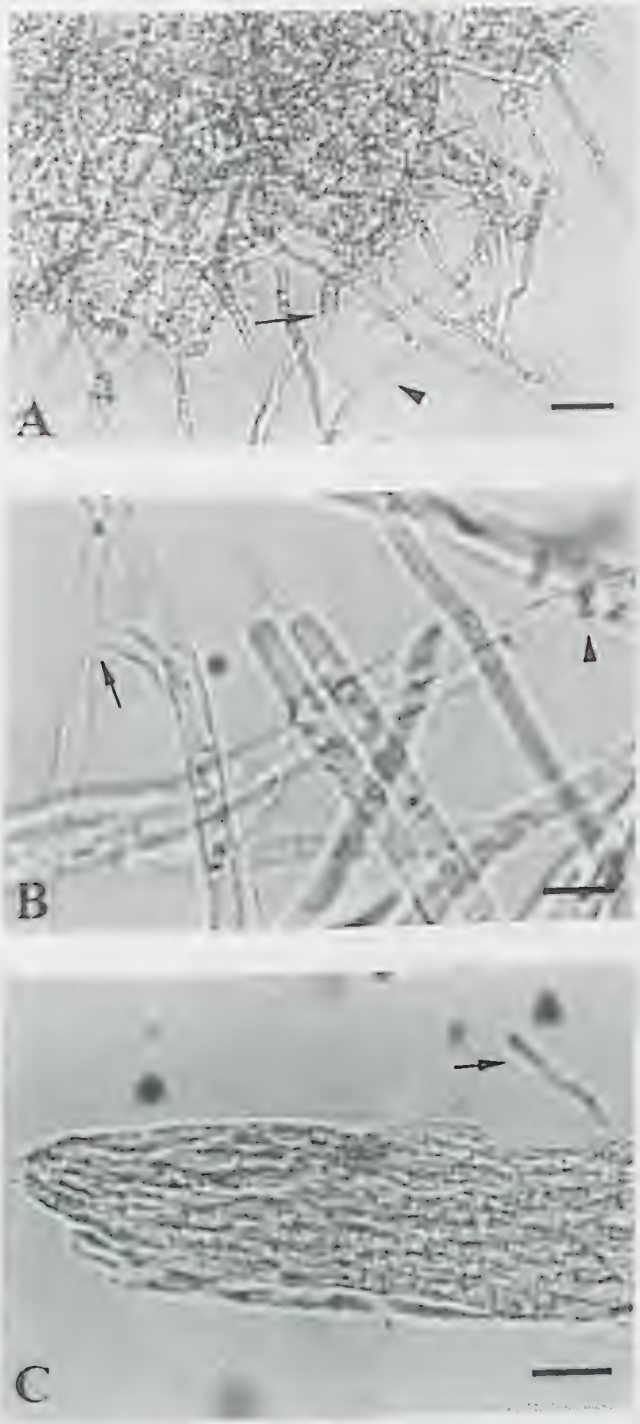

Figure 3 Fungal isolates. Septate (arrow head), branched mycelium (arrow) from a colony with fine aerial hyphae (A) and lateral hyphae from a stout, erect thallus (B). Refractile droplets are denoted with a r. Phase contrast. Bars = 10 µm and 4 µm, respectively. C. Thallus from a colony with stout, erect aerial hyphae. A single lateral hypha projects from the main thallus (arrow). Phase contrast. Bar = 16 µm.

Results

Abalone

Abalone lengths ranged from 102.1 to 153.8 mm for *H. iris* and 70.8 to 110.3 mm for *H. australis*. Prevalence of shell mycosis differed between species. However, the difference was not significant ($p>0.05$; $N=17$). Four of seventeen *H. iris* had moderate (2+) to advanced lesions (3+), and ten of seventeen *H. australis* were infected with early (1+) to advanced (3+) lesions. We observed no significant relationship between shell length and incidence of shell mycosis ($p=0.534$ and $p=0.242$, respectively; $N=17$ each). The average condition index (CI) of *Haliotis iris* (0.689) differed from that of *H. australis* (0.762) ($p<0.0001$, $N=17$). However, abalone CI was not influenced by the shell mycosis (*H. iris*: $p=0.240$, $t=-1.23$, $DF=14$; *H. australis*: $p=0.967$, $t=-0.0417$, $DF=14$).

We observed a significant relationship between numbers of circulating haemocytes and degree of shell mycosis ($p<0.05$, $N=15$) in *H. australis* but not in *H. iris* ($p>0.05$, $N=14$). The number of circulating

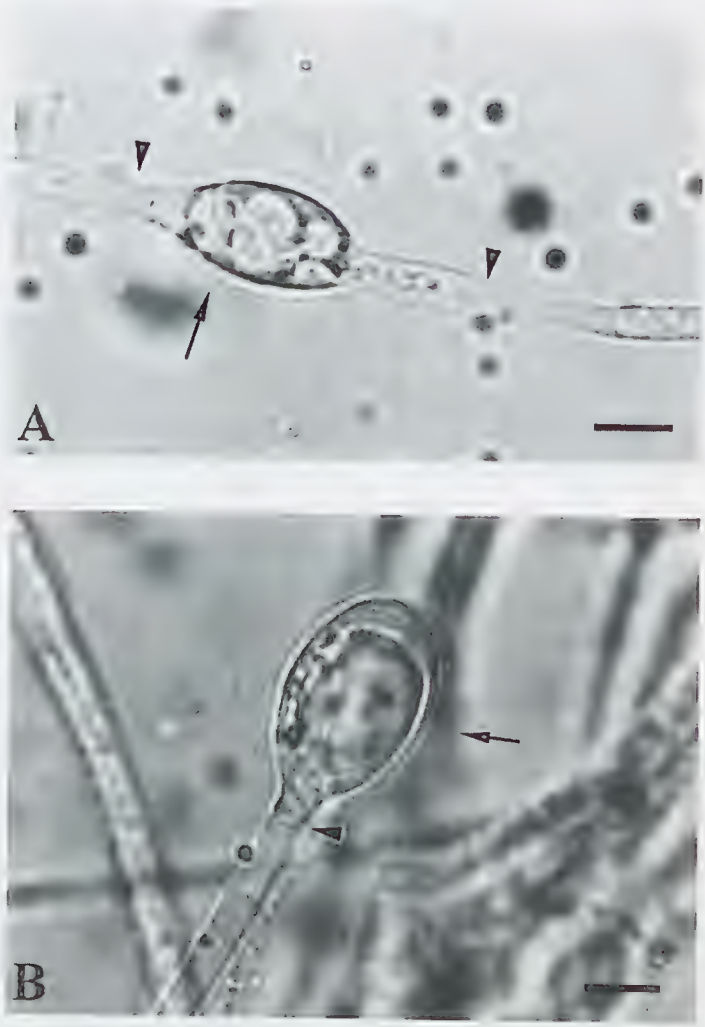

Figure 4 Chlamydospores from a fungal isolate (arrow). Note that the chlamydospore is located between septa (arrow heads, A) or apical to a septum (arrow head, B). Phase contrast. Bars = 4 μm.

haemocytes was elevated in infected *H. australis* (1.19 x 107 cells/ml) relative to uninfected individuals (6.72 x 10^6 cells/ml). No significant relationships were observed between the degree of fungal infection and protein levels of whole hemolymph, serum or sex (immature, male or female) (Spearman Rank Order Correlation: $p<0.050$).

Numerous spherical and polygonal bodies were observed in advanced lesions; many contained fungal hyphae (Figure 1a). We observed numerous sparsely branched, septate fungal hyphae that contained refractile droplets in fresh preparations of both newly established and advanced shell lesions (Figure 1b). Hyphae measured 1.20 – 2.38 µm ($\bar{X}$ = 1.83 µm) in diameter. Occasionally, wider hyphae that measured 2.38 – 3.60 µm ($\bar{X}$ = 2.60 µm) in diameter with an apical swelling that

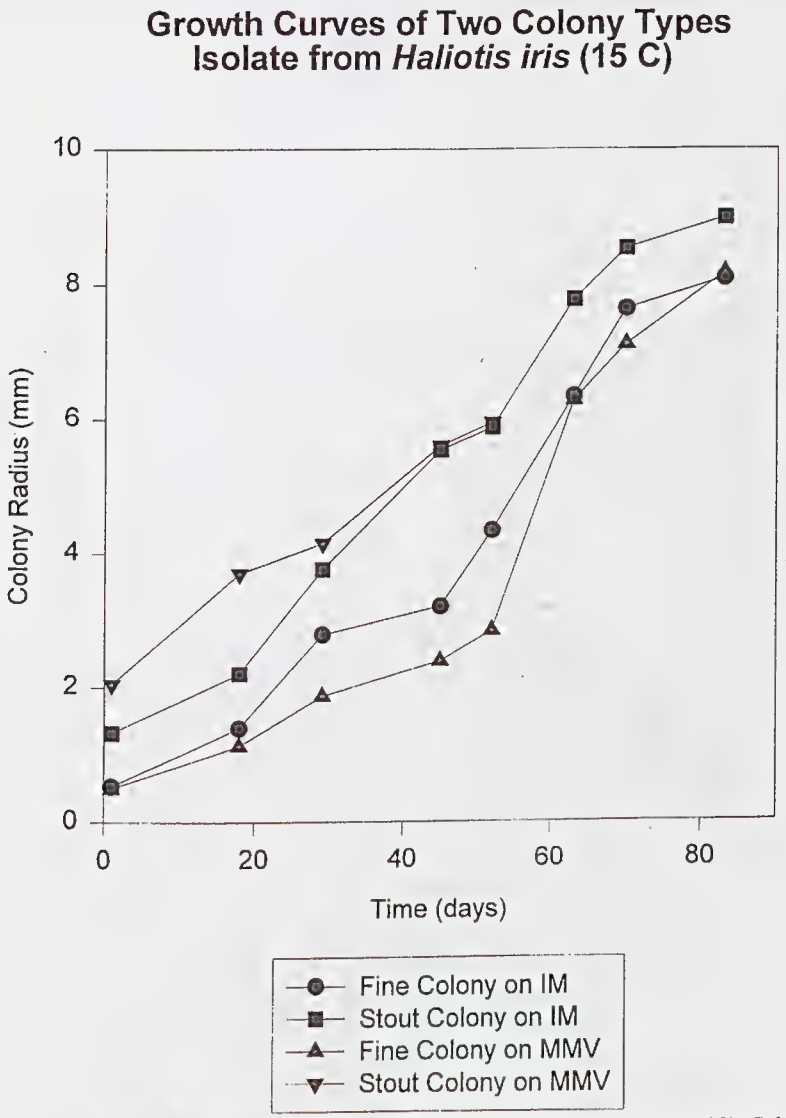

Figure 5 Growth curves of two colony types isolated from an *Haliotis iris* shell lesion (15 °C). Colonies with fine aerial hyphae on IM agar (●) and MMV agar (▲). Colonies with stout, erect aerial hyphae on IM agar (■) and MMV agar (▼).

measured 4.80 – 7.20 x 7.20 – 15.00 µm ($\bar{X}$ = 6.20 x 11.80 µm) were observed within lesions (Figure 1c). Visual examination of the shell, mantle and foot adjacent to lesions showed that the fungi and deposition of conchiolin were limited to the shell (See Grindley *et al.* Submitted for histological examination). Although, *Polydora* sp. infested animals used in our study, no tubes penetrated through to the inner shell surface and none were observed in association with the shell lesions.

Fungal Isolation

Visible growth of aerial hyphae was observed on plates within 12 d at 16 °C on both MMV and IM agars only. Three morphologically indistinguishable isolates were obtained: two originated from

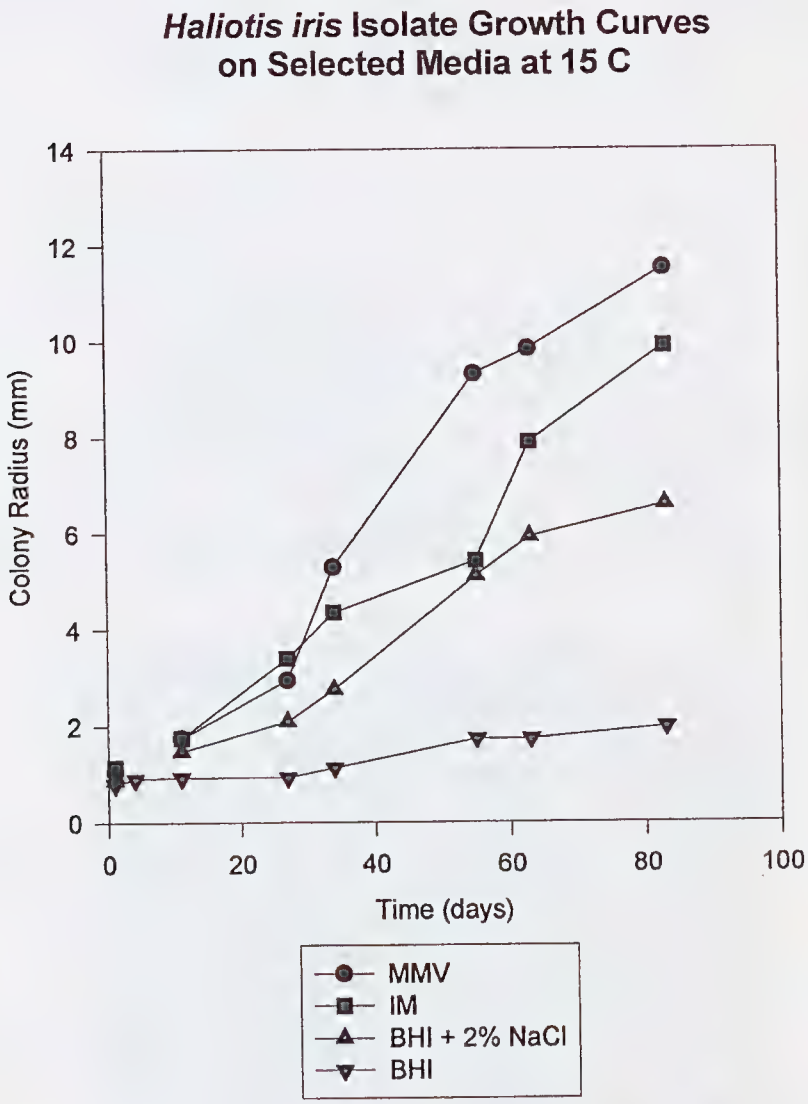

Figure 6 Growth curves of *Haliotis iris* isolate on four media at 15 °C: MMV (●), IM (■), BHI + 2% NaCl (▲), and BHI (▼).

Haliotis iris shells (Hi and DL) and one was cultured from an *H. australis* shell. Unfortunately, the isolate from *H. australis* was lost during passage. Colonies were white to grey in colour with an irregular rhizoid margin. Aerial hyphae were fine to stout and irregular with tapering tips. Fine lateral hyphae projected distally from the primary structure. After passage in the laboratory, colony morphology of both isolates developed into two distinct forms: those with fine aerial hyphae and those with stout, erect, aerial hyphae (Figure 2). Individual colony morphology was retained upon subsequent passage. Fine aerial hyphae were sparsely branched, septate, measured 1.24 – 3.18 µm ($\bar{X}$ = 1.38 µm) in diameter, and contained numerous refractile droplets (Figure 3). Stout aerial thalli, measured 197 – 264 µm at the base and tapered to 2.4 – 7.2 µm at the tip. Lateral mycelia that projected from the stout, erect thalli, measured 1.4 – 4.65 µm ($\bar{X}$ = 2.38 µm) in diameter and were septate, sparsely branched and contained refractile droplets (Figure 3). Chlamydospores formed as

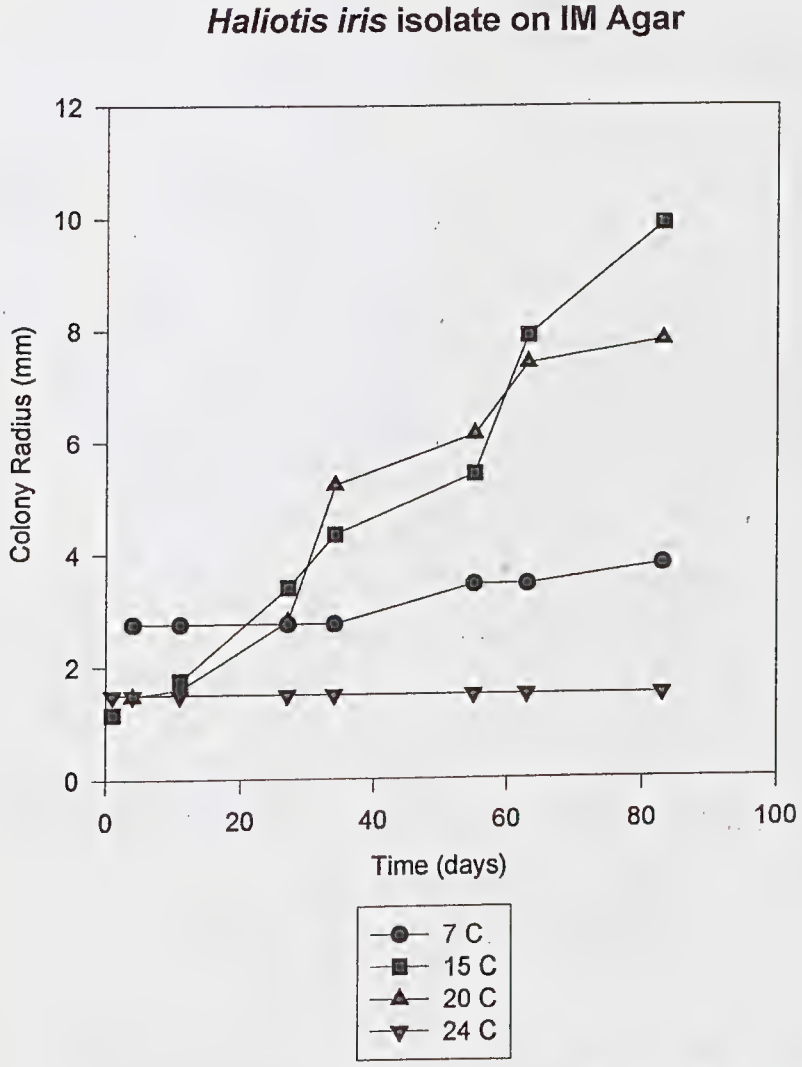

Figure 7 Differential growth of *Haliotis iris* isolate at four temperatures: 7 °C (●), 15 °C (■), 20 °C (▲), and 24 °C (▼).

terminal or intercalary cells, 6.4 μm diameter with refractile bodies, and were observed infrequently upon microscopic examination of both colony types (Figure 4).

Fungal Growth Characteristics

Mycelial growth of both colony types was similar as illustrated in Figure 5. Although both colony types appeared to grow equally well on IM and MMV (Figure 5), repeated examination indicated that the fungus grew best on MMV, followed by IM. Mycelial growth was moderate on BHI+ 2% NaCl and poor on BHI without added salt (Figure 6). Mycelial growth was also poor on CMA and PDA. The fungus grew best at 15 °C, moderate at 19 °C and poor at 7 °C. No growth was observed at 24 °C (Figure 7). All attempts to induce sporulation have failed.

Discussion

We report the first isolation of a fungal pathogen from shell lesions of abalone, *Haliotis* spp. in New Zealand. Shell lesions similar to those described herein have been reported in *H. iris* from New Zealand by Sinclair (1963) and Sainsbury (1977). Sinclair (1963) observed tubercles on the nacreous layer of shells that were composed of alternating layers of conchiolin and nacre and suggested that the lesions were caused by irritation from either sand particles or *Polydora* sp. tubes. Sainsbury (1977) also noted blisters on the nacreous layers of many abalone shells in the Banks Peninsula, New Zealand. Lesions in some animals filled the entire inner surface of the shell and resulted in loss of the shell. Lesions in these animals were thought to be caused by irritation due to boring organisms and/or sediment lodged between the shell and mantle. We did not observe sand particles or *Polydora* tubes in the area of the lesions. In addition, the lesions observed in our study were composed entirely of conchiolin and fungal hyphae and, hence, differed from those reported by Sinclair (1963). Neither Sinclair (1963) nor Sainsbury (1977) reported microscopic examination of shell lesions, particularly early soft lesions as used in our study. Thus, the authors may have overlooked and alternative irritation such as the fungus we consistently observed in all lesions examined.

Fungal lesions in shells of *Haliotis iris*, *H. australis* and *H. virginia* in New Zealand were initially thought to result in decreased value of abalone shells, without an effect on the survival of the animals. However, abalone culturists have experienced losses of farmed animals with lesions while unaffected abalone survived (Grindley 1994). Stress associated with handling and intensive culture conditions may lead to mortality of abalone with shell mycosis as evidenced by observations of Sainsbury (1977) and Grindley *et al.* (Submitted). Although we observed no visual evidence of fungal penetration into live tissues and no differences in condition indices of affected versus unaffected animals, we did observe a significant increase in the number of circulating haemocytes in affected relative to unaffected *H. australis*. The causal relationship between hemocyte numbers and degree of shell mycosis is unclear but may indicate stress or an early response to the disease. The small number of *H. iris* that were infected (N=4) may have attributed to the lack of association between degree of fungal infection and density of circulating haemocytes in this species. An understanding of the pathogenesis of and possible treatments for the shell mycosis is needed.

Fungal-induced shell lesions have been reported in bivalves for several decades (Korringa 1951, Alderman 1985). Ostracoblabe implexa (Class Zygomycotina; Phycomycetes) is the causative agent of shell disease or 'Maladie du pied' of oysters and cockles in Europe and North America. The disease is associated with sea water temperatures above 20 °C and is characterized by the formation of small white spots on the inner surface of the shell that develop into brown warts due to accelerated deposition of conchiolin. Similar to early reports of abalone shell mycosis described herein, definitive proof that the shell disease results in mortality is lacking (Sainsbury 1977; Elston 1994).

Our observation of morphologically similar hyphae in fresh squashes of lesions and the isolation of morphologically indistinguishable colonies from two animals that differentiated into two distinct colony types (stout and fine) suggests that we have isolated a pleomorphic fungus. In addition,

growth characteristics of both colony types was similar as depicted in Figure 5 and fungal morphology is known to change with differing culture conditions (Morrin and Ward 1990; Tucker and Thomas 1994). These data also suggest that the two colony types may be different phenotypes of the same organism. However, all isolates were handled in the same manner in this study and, thus, the possibility exists that the two colony types represent different fungi. Despite this, both colony types were isolated in pure culture from shell lesions of two different abalone in this study which suggests that an association exists between both colony types and abalone shell lesions. *Atkinsiella awabi* (Class Zygomycotina; Phycomycetes) occurs in tissue lesions of stocked abalone, *Haliotis sieboldii*, in Japan. The fungus we isolated from shell lesions differed in nearly all characteristics from *A.* awabi as described by Kitanchareon *et al.* (1994). *Atkinsiella awabi* is a nonseptate fungus, with an irregular thallus, and is larger in diameter than the fungus reported herein (16–41 µm versus 1.24–4.65 µm, respectively). In addition, *A. awabi* readily sporulates, grows best at 20 °C and grows equally well on CMA and a medium similar to MMV. In contrast, the fungus we isolated is septate with a regular thallus, does not readily sporulate, grows best at 15 °C and growth on MMV far exceeds that on CMA. However, both organisms are branched and require salt for growth. The taxonomic placement of the fungus reported herein is uncertain. Due to lack of sexual structures, the fungus reported herein is provisionally placed in the Class Deuteromycotina (Fungi Imperfecti, Jawetz *et al.* 1987). Morphological characteristics of the vegetative mycelium cultured from shell lesions resembled those of hyphae within lesions (Figures 2 & 4) and suggests that the fungus described in our study is the causative agent of the shell lesions. We are currently attempting to reproduce shell lesions in abalone in our laboratory.

Acknowledgments

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